DOCKET NO.: CARP0015-100 **PATENT**
APPLICATION SERIAL NO. 10/693,308

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Frank Grosveld **Confirmation No. 1498**

Serial No.: 10/693,308 **Art Unit: 1632**

Filing Date: October 24, 2003 **Examiner: Anoop K. Singh**

For: IMMUNOGLOBULIN 2

Customer No.: 34132

DECLARATION OF LOUIS M. WEINER, M.D.

1. I have been engaged by Erasmus University to provide my opinion on certain issues concerning the above-identified application. I am being compensated at a rate of $ 450 per hour to do so.
2. My *curriculum vitae* ("cv") is attached as Exhibit A.
3. I received a Bachelor of Arts, cum laude, honors in biology, from the University of Pennsylvania in 1973. I received my medical degree from the Mount Sinai School of Medicine, N.Y., N.Y. in 1977.
4. I am currently the Vice President, Translational Research and Chairman, Department of Medical Oncology at the Fox Chase Cancer Center ("Fox Chase"), Philadelphia, Pennsylvania. I have been with Fox Chase since 1984. I have been Chairman of the Department of Medical Oncology since 1994; I have been Vice President, Translational Research since 2002.
5. As Vice President of Translational Research, my duties includeconnecting laboratory science with clinical investigation to improve the diagnosis and therapy of cancer. I oversee the allocation of research funds to support novel technology development and to stimulate collaborations among bench researchers and clinical investigators.
6. As is evident from my CV, I have been involved in immunology-focused research throughout my 30-year career, and have published extensively on immunology-focused

topics. I am a manuscript reviewer on numerous peer-reviewed journals including, for example, *Journal of Immunotherapy*, *Nature Biotechnology*, and *Science*. I have been an invited speaker at conferences regarding the protein engineering of monoclonal antibodies.

7. I am also the inventor on several patent applications, including U.S. Patent Publication No. 20040071696 involving bispecific antibodies.

8. I have reviewed the above-identified application. The correspondence with the examiner and the recent response from the inventor Dr. Grosveld. I am familiar with the involved technology, namely single heavy chain antibodies. Single heavy chain antibodies are antibodies composed of heavy chains only; no light chains are present. Functional heavy chain only antibodies are naturally produced by the camelid family and were first described by Hamers-Castermans in 1993 (Nature 363,446-448) **(copy attached)**. Camelid VHH heavy chain only antibodies are unable to bind light chain due to the absence of the CH1 domain.

9. As single heavy chain antibodies are smaller in size than antibodies comprising full-length heavy and light chains, there has been much interest generated in their use for therapy, in particular the VHH antigen binding domain. Accordingly, there has been much interest in developing antibodies against specific antigens for administration to mammals other than camelids including, of course, humans. Camelid antibodies are likely to produce an immune response in man, so either camelid antibodies must be "humanized" after isolation from the camelid, or alternative routes devised to generate human or "camelised" human heavy chain only antibodies more suitable for human therapy.

10. The present application reports, and claims, methods for producing single heavy chain antibodies in transgenic non-human mammals using the mouse as a model system. A immunoglobulin locus heavy chain locus engineered to prevent CH1 expression is introduced into the germ-line using well established technology. Heavy chain only antibody is produced in a B-cell specific manner as expected in response to antigen challenge.

11. To produce the antibodies, a transgene comprising a heavy chain locus comprising V, D

and J segments and heavy chain constant regions devoid of CH1 is expressed in a non-human mammal. I understand that one requirement, as recited in the claims, is that the heavy chain locus comprises at least one V exon, at least one D exon, and at least one J exon, capable of recombining to form VDJ coding sequences. The ability of the V, D, and J exons to recombine is extremely important, as this is one of the mechanisms that facilitate diversity in naturally produced antibodies . Another requirement, as recited in the claims, is that the heavy chain locus be expressed in B cells such that VDJ rearrangement occurs in specialized antibody producing cells. Another requirement of the invention, as recited in the claims, is that the heavy chain locus, when expressed, does not express a functional CH1 domain as part of the constant region.

12. All claims of the above-identified application are currently under rejection by the U.S. Patent & Trademark Office ("the Office"). I have read the Final Rejection dated February 12, 2007. I have also read the Interview Summary dated June 1, 2007; the Request for Reconsideration ("Request") filed June 25, 2007; and the Advisory Action Before the Filing of an Appeal Brief ("Advisory Action") dated July 9, 2007.

13. In the Final Rejection, the Office rejected the claims under the following bases:

    1. for lack of enablement under 35 USC § 112, first paragraph;
    2. for being incomplete as omitting essential steps under 35 USC § 112, second paragraph;
    3. as anticipated by Ledbetter et al (WO 99/42077) under USC § 102(b); and
    4. for obviousness-type double patenting over the claims of copending Application No. 10/692,918.

    I have been asked to address bases 1 and 3 above.

14. The first basis for rejection is further broken done into four issues. I will address the first three issues. The first issue appears to be that the specification only provides prophetic reference to most of the method steps, and does not provide any sequence information for the vectors (see page 6 of the Final Rejection). The Office's view is that undue experimentation would be required to practice the invention. I respectfully disagree.

15. I have read Janssens et al., *PNAS,* USA, 103(41)15130-15135, October 10, 2006. It is clear from the successful experiments reported in Janssens et al., which were performed

using the methods provided in the above-identified application, that the above-identified application provides sufficient detail for one of skill in the art to practice the invention.

16. The Office acknowledges the disclosure in Janssens et al. in the Advisory Action, but is limiting its effect. The Office seems to be taking the position that Janssens et al. is *only* enabling for expression of the GΔ construct (IgG heavy chain) in a wild type background, and is *only* enabling for the production of antibody in response to antigen challenge in GΔ construct-containing mutant mouse. The Office states that the guidance provided by applicants invites others to try different existing transgenic knockout nonhuman animals incapable of producing antibodies that include light chains. (See the Advisory Action, page 4.) The Office provides no scientific reason to conclude that the MΔGΔ construct, which Janssens et al. reports was expressed in the mutant mouse, would not also be expressed in wild type mouse. The Office also provides no scientific reason to conclude that the production of antibody in response to antigen challenge can only occur in mutant mice that do not produce light chain-containing antibodies. Regarding the first conclusion, I note that Janssens et al. report that the mutant mice produced some IgG, IgA, and IgE, i.e., antibodies that include light chains (p. 15130, col. 2, first full paragraph). Regarding the second conclusion, camelids produce both single heavy chain only antibodies and antibodies that include light chains, so it is clearly not necessary that the mammals be mutants that do not produce light-chain containing antibodies. As Janssens et al. confirms, the key components necessary for practicing the invention are set forth in the specification; the gene sequences introduced comprise all the necessary features required for the productive expression of heavy chain-only antibody (devoid of light chain) derived from heavy chain-only loci in the B-cells of transgenic mammals. The additional statement and data provided by Dr. Grosveld confirms this view. The data show that the MΔGΔ locus is expressed in wt mice and that human IgM is produced.

17. While the presence of suppressed endogenous heavy and light chain genes may be advantageous when selecting for antigen specific heavy chain-only antibodies against a background of endogenous immunoglobulin gene activity, it is clear that heavy chain only transgenes compete **successfully** with the endogenous genes active in the same B-cells, and allelic exclusion determines which loci will ultimately be used to express

functional antibody in the activated B-cell. Further, Janssens et al have shown that a camelised human locus comprising camelid VHH segments plus Human D and J segments plus human constant regions are functional and that camelised human heavy chain only antibody (IgM and/or IgG) circulates in plasma in genetic backgrounds where the endogenous mouse immunoglobulin genes are active, and that the same camelised human heavy chain only locus functions in a background (μMT) where endogenous mouse immunoglobulin gene activity has been suppressed. Moreover when the camelid VHH segments are replaced with human V segments, then fully human heavy chain only antibodies are produced in a wild type mouse background where the mouse heavy and light chain loci remain functional (See paragraph 5 of Dr. Grosveld's declaration, which I understand is being submitted concurrently.) The genetic background thus has no bearing on the enablement of this invention.

18. The second issue is that the production of functional camelid heavy chain is unpredictable. The Office cites a recently published reference for support of its position -- DeGenst et al, *Dev. Comp. Immunol.*, 30(1-2):187-98 (2006). The Office states that the reference reports that the timing and actual mechanism of class switching from mu to the dedicated gamma isotype in camelids remains elusive (see page 7 of the Final Rejection). Class-switching, however, is not a requirement of the method of the present invention. As Janssens et al. reports, the GΔ construct, which contains no mu exons, was successfully expressed (p. 15132). The Office also states that it is "not apparent from the specification whether a method as recited in claims would result in fully functional scIgG molecule" (see page 7 of the Final Rejection). Janssens et al., however, shows functional expression of both mu and gamma single heavy chain isotypes (Janssens et al Figure 6; three for IgM, many more for IgG, and all of these bind antigen, pp. 15132 and 15134).

19. The present invention is about the derivation of heavy chain only antibody in the B-cells of transgenic mammals. The inventor has defined the gene sequences to be introduced and has demonstrated that, provided the CH1 region is not expressed in the transgene(s), functional antibody is produced of the class or classes determined by the effector regions present in the transgene. This has been exemplified through the expression of heavy chain only IgM and IgG subtypes, or IgG subtypes alone, in different transgenic lines. It

is also clear that this is independent of the presence or otherwise of a functional endogenous immunoglobulin locus.

20. The third issue is that the claims cover the use of any promoter, with any regulatory elements, in any non-human transgenic mammal. The Office alleges that, at the time of invention (which I understand to be at least April 24, 2001, the earliest priority date claimed), only the mouse was recognized by the art of record as a routinely manipulated animal, and that the art of record recognized the unpredictability of making transgenic animals other than mice (see page 8 of the Final Rejection). I do not agree that the art of record shows this. The Office is focusing upon levels of expression sufficient to show a particular phenotype. In the present invention, it is only important that heavy chain-only antibody expression occurs in B-cells. All that is required is sufficient gene expression to isolate the antibody mRNA or antibody. As reported in Janssens et al., antibodies can be isolated and subsequently characterised by hybridoma production or phage display libraries (p. 15133). Dr. Grosveld's declaration shows that human heavy chain-only antibody mRNA can be isolated and sequenced from the B-cells present in a few microlitres of blood, and this against a background of B-cells producing endogenous mouse antibody. Similarly, human heavy chain-only antibody can be identified circulating in plasma.

21. Moreover, the techniques used (i.e., PCR amplification of mRNA and DNA sequencing; antibody detection by Western blotting) were routine laboratory tools at the priority date. The invention results in the production of novel antibodies. Once identified and characterized, heavy chain only antibodies or fragments thereof can be amplified and manufactured using established technologies of no relevance to this invention.

22. The second basis for rejection that I was asked to consider was the rejection for anticipation over Ledbetter et al. In the Final Rejection, the Office states that Ledbetter et al. anticipates claims 1 and 2. I am aware that, in order for a reference to anticipate a claim, it must disclose each and every limitation of the claim, and must be enabling. Specifically, the Office states that Ledbetter et al. discloses a method to produce VHH monoclonal and polyclonal antibodies in a transgenic animal in response to antigen challenge, citing page 33, para. 2-3 and page 34, para. 2 of Ledbetter et al. The Office

further states that, since Ledbetter et al. contemplated producing monoclonal antibody by immunizing the transgenic mice, expression of the VHH heavy chain locus in B cells in response to antigen challenge is inherent. (See Final Rejection, page 15.) I have reviewed Ledbetter et al. and disagree with the Office's assessment.

23. The concept of transgenic animals expressing llama VHH is first raised on p. 32 of Ledbetter et al. and refers to "the VHH sequences isolated by the methods disclosed herein." These are VHH binding domains derived from camelid mRNA and so are a result of transcription from already rearranged llama genes (see p. 44-46 section 8.1.2). Each VHH binding domain will comprise a VHH gene segment and D gene segment and J gene segment fused together as a single transcription unit. On p. 33, line 22, of Ledbetter et al., it is proposed that transgenic mice expressing VH binding domains "maybe used for the production of VHH to any antigen by immunizing transgenic animals with an antigen". This, however, is technically impossible -- the VHH binding domains are transcribed from already rearranged llama genes.

24. As is clear from the above, V, D, J rearrangement, and subsequent affinity maturation, occurs in the camel. This follows the teaching of Hamers-Casterman et al., referenced above, who first described camelid heavy chain only antibodies, and whose work is the subject of many published US patents describing the generation and subsequent use of camelid antibodies. See, for example, U.S. Patent Nos. 6,838,254; 6,765,087; 6,015,695; 6,005,079; 5,874,541; 5,840,526; 5,800,988; and 5,759,808. Ledbetter et al. then isolates a rearranged camelid gene (See Fig. 4) and proposes to reintroduce this into a transgenic mouse for the purpose of raising further novel antibodies in response to further antigen challenge. Such a gene, however, lacks all the features necessary for antibody diversity (e.g., multiple D and J regions) and cannot rearrange as it has been rearranged already. (See attached figure depicting the differences between Ledbetter et al. and the present invention.) Moreover, a recent publication from an independent laboratory has shown that, while such a rearranged gene can be expressed productively as a transgene in a mouse background (B-cell activation occurs) the resulting antibody retains all the features of the antibody originating from the camel (see Zou et al., "Expression of a dromedary heavy chain-only antibody and B cell development in the mouse," *J Immunol.*

2005 Sep 15;175(6):3769-79 **(copy attached)**). Thus, Ledbetter et al. fails to describe or generate a transgenic animal capable of producing heavy chain only antibody in response to antigen challenge.

25. As I understand it, claims 1 and 2 of the above-identified application are claiming the production of a single heavy chain antibody having a camelid (VHH) variable region. The antibody is produced in response to challenge with a particular antigen and, thus, binds to that antigen. Janssens et al. reports that single heavy chain antibodies against *E.* coli hsp70, *Bordetella pertussis, Tetanus* toxoid, rtTA, and TNFα were obtained using the method of the invention (p. 15133, col. 2, and p. 15135, col. 1). In contrast, Ledbetter et al. does not disclose a method to produce VHH monoclonal and polyclonal antibodies in response to antigen challenge. Ledbetter generates camelid VHH antibodies in llama and then displays recovered VHH binding domains by phage display (see Figure 1, **attached**). Accordingly, Ledbetter et al. does not enable the production of antibody in response to antigen challenge from a heavy chain only locus in a transgenic animal and, thus, cannot anticipate claims 1 and 2.

26. I hereby declare that all statements made herein are of my own knowledge true and statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

11/15/07 _____

Date Dr. Louis M. Weiner

CURRICULUM VITAE

Updated 08/07

Louis M. Weiner, M.D.

NAME: Louis Marc Weiner

DATE OF BIRTH: May 21, 1951 - Philadelphia, PA

EDUCATION:

1973	University of Pennsylvania, Phila., PA.	B.A. cum laude with Honors in Biology
1977	Mount Sinai School of Medicine, N.Y., N.Y.	M.D.

CLINICAL TRAINING:

1977-1978	Medical Center Hospital of Vermont University of Vermont, Burlington, VT	Intern, Dept. of Medicine
1978-1980	Medical Center Hospital of Vermont University of Vermont, Burlington, VT	Resident, Dept. of Medicine
1980-1981	Medical Center Hospital of Vermont University of Vermont, Burlington, VT	Chief Resident, Dept. of Medicine
1981-1982	New England Medical Center Hospital Tufts University School of Medicine Boston, MA	Clinical Fellow, Hematology/Oncology
1982-1984	New England Medical Center Hospital Tufts University School of Medicine Boston, MA	Research Fellow, Hematology/Oncology

FACULTY POSTS:

Current:	Vice President, Translational Research Chairman, Department of Medical Oncology G. Morris Dorrance, Jr. Endowed Chair in Medical Science Senior Member, Division of Medical Sciences Fox Chase Cancer Center
1980-1981	Attending Physician, Department of Medicine, Medical Center Hospital of Vermont, Burlington, Vermont
1980-1981	Instructor, Department of Medicine, University of Vermont College of Medicine, Burlington, Vermont
1984-1990	Associate Member, Department of Medical Oncology Fox Chase Cancer Center, Philadelphia, PA

1985-1994	Director, Medical Oncology Fellowship Program, Fox Chase Cancer Center
1987-1990	Assistant Professor, Temple University School of Medicine
1991-1995	Member, Department of Medical Oncology Fox Chase Cancer Center, Philadelphia, PA
1991-1995	Associate Professor, Temple University School of Medicine
1994-Pres	Chairman, Department of Medical Oncology Fox Chase Cancer Center, Philadelphia, PA
1995-Pres	Senior Member, Department of Medical Oncology Fox Chase Cancer Center, Philadelphia, PA
1995-Pres	Professor, Temple University School of Medicine
1996-Pres	Director, Developmental Therapeutics Program Fox Chase Cancer Center, Philadelphia, PA
1998-2005	Chief, Section of Medical Oncology Department of Medicine, Temple University Hospital
2000-Pres	Consulting Staff, Division of Medical Oncology Department of Medicine, Jeanes Hospital
2002-Pres	Vice President, Translational Research Fox Chase Cancer Center

BOARD CERTIFIED:

1978	National Board of Medical Examiners
1980	American Board of Internal Medicine
1985	Medical Oncology

LICENSURE: Pennsylvania - #MD 031312E

AWARDS:

1985	American Cancer Society Career Development Award
1986	National Cancer Institute Clinical Investigator Award
1997	Janssen Pharmaceutical Research Foundation: Research Award for Outstanding Contributions to the Field of Receptor Targeted Therapy
2001	American Cancer Society-Southeast Region Scientific Research Award
2002	American Association for Cancer Research Scientist <-> Survivor Program Award
2002	G. Morris Dorrance, Jr. Endowed Chair in Medical Science
2000-2006	Best Doctors in America
2002-2005	Who's Who in Medicine and Healthcare
2002; 2004-2007	Philadelphia Magazine's Top Docs
2007	Elliott Osserman Award for Distinguished Service in Support of Cancer Research; Israel Cancer Research Foundation (ICRF) Review Panel 2005-2007

EDITORIAL BOARDS:

1996-	Editorial Board: Journal of Immunotherapy
1996-2000	Editor-in-Chief: Presentations in Focus/Oncology Forum
1996-	Tumor Board: Oncology Times
1998-	Editorial Board: Journal of the Int'l Society of Tumour Targeting
1999-	Editorial Board: Clinical Cancer Research
1999-	Editorial Board: Investigational New Drugs
2000-2002	Editorial Board: Journal of Clinical Oncology
2001-	Editorial Advisory Panel: Expert Review of Anticancer Therapy
2000-	Associate Editor: Cancer Research
2001-2002	Editor-in-Chief: PeerView in Focus
2001-2006	Editorial Board: Molecular Cancer Therapeutics
2001-	Editorial Board: Human Antibodies
2002-	Editorial Board: International Journal of Oncology
2003-	Editorial Board: In Vivo
2003-	Editorial Board: Advances in Targeted Cancer Therapies
2005-	Editorial Advisory Board: Medscape Hematology-Oncology Medicine

PEER-REVIEWED RESEARCH SUPPORT:

1986 National Cancer Institute - Clinical Investigator Award KO8 CA01130. - Principal Investigator 1986-1989

1989 National Cancer Institute - RO1-CA50633. "Antibody-Targeted Immunotherapy of Cancer." - Principal Investigator: 1989-2004

1990 National Cancer Institute-U01-CA51880 National Cooperative Drug Discovery Group. "Engineered Antibreast Cancer Single-Chain Fv Immunotoxin." - Program Leader 1990-1995

1992 Division of Cancer Treatment, NCI - Master Agreement.
RFP No. NCI-CM-27732-49, "Clinical Evaluation of Biological Response Modifiers (BRMs) for the Treatment of Cancer". - Principal Investigator.
NO1-CM-27732-01, "Phase I Evaluation of the Murine Bispecific Monoclonal Antibody 2B1" 1992 - 1993

1993 National Cancer Institute - U01 CA58262 - "Clinical Development of 2B1 Bispecific Monoclonal Antibody" Principal Investigator: 1993- 1997

1993 National Cancer Institute - K12 CA01728 - "Clinical Oncology Research Career Development Program" Principal Investigator: 1997-2003

1994 National Cancer Institute - U10 CA27525 - "Eastern Cooperative Oncology Group" Principal Investigator, Fox Chase Cancer Center: 1994-2004

1996 National Cancer Institute - R01 CA65559 - "Tumor Targeting by Single-Chain Fv Molecules" Principal Investigator: 1996-2003

1998 ARMY IDEA – DAMD17-98-1-8084 - "Fcγ Receptor-targeted Immunization for Breast Cancer" Principal Investigator 1998-2001

1998 ARMY Clinical Translational – DAMD17-98-1-8307 - "Targeting Breast Cancer with Anti-HER2/neu Diabodies" Principal Investigator 1998-2003

1999 ARMY – DAMD17-99-1-9183 – "Antibody-Pretargeted Cytokine Therapy of Cancer" Principal Investigator 1999-2003

2002 National Cancer Institute – R21 CA097461 – "Tumor and Endothelial Cells as Drug Targets in Blood" Principal Investigator 2002-2005

2003 National Cancer Institute – R41 CA099410 – "Gene Expression in Circulating Tumor Cells" Principal Investigator 2003-2004

2003 National Cancer Institute - RO1-CA50633-18. "Antibody-Targeted NK Cell Activation for Cancer." Principal Investigator: 1989-2008

2003 National Cancer Institute – K08 CA090468 – "Therapeutic Inhibition of Firbrblast Activation Protein 2003-2008

2004 National Cancer Institute – P50 CA083638 – "SPORE in Ovarian Cancer" - Co-Principal Investigator 2004-2009

2005 National Cancer Institute – P30 CA006927 – "Comprehensive Cancer Center Program at Fox Chase" Senior Leader and Major Program Leader 2005-2010

2005 National Cancer Institute – R42 CA099410 – "Gene Expression in Circulating Tumor Cells" Co-Principal Investigator 2005-2007

2006 National Cancer Institute – S10 RR019008 – "Guava EasyCyte Base System (Guava Personal Cell Analysis-96 System) 2006-2007

2006 National Cancer Institute – R01 CA107088 – "Bispecific Antibody Pretargeting for Therapy" 2006-2010

2006 National Cancer Institute – R21 CA121541 – "Analysis of Circulating Tumor Cells in a Phase I/II Study for Breast Cancer" 2006-2008

2006 American Cancer Society – MRSG-06-003-01-CCE "Circulating Tumor Cells to Develop Novel Pancreatic Cancer Therapy" 2006-2010

2006 National Cancer Institute – R01 CA121033-01 – "Adaptive Immunity from High Affinity Anti-HER2/neu Monoclonal Antibodies" Principal Investigator – 2006-2011

OTHER RESEARCH SUPPORT:

1985 American Cancer Society Career Development Award - Principal Investigator

1985 Biogen Corporation - "A Phase III Study Comparing Therapy Using Recombinant Interferon-gamma with Depoprovera in Advanced Renal Cell Carcinoma." - Co-principal Investigator

1986 Frank Strick Foundation - "Biological Therapy Support Facility Development" - Principal Investigator

1986 Mary L. Smith Charitable Lead Trust - "Development of Laboratory Models for Evaluating the Clinical Potential of Biologic Response Modifier Combinations" - Co-principal Investigator

1986 Benjamin Franklin Partnership - "Preclinical Evaluation of Magnetite-Antibody Congeners for Immunodiagnosis and Immunotherapy of Human Neoplasms" - Co-principal Investigator

1987 Frank Strick Foundation - "Development of Laboratory Models for Studying Ex Vivo Activation of Human Effector cells to Potentiate Monoclonal Antibody Therapy" - Principal Investigator

1987 Cetus Corporation - "Phase I Trial of 260F9-MAb-rRA Immunotoxin" - Principal Investigator

1991 Frank Strick Foundation - Clinical Development of Bispecific Monoclonal Antibodies - Principal Investigator

1991 Genentech - "Phase I Trial of M-CSF and γ-Interferon" - Principal Investigator

1991 Genetics Institute - "Phase I Trial of M-CSF and γ-Interferon" - Principal Investigator

1992 Frank Strick Foundation - "Clinical Development of Bispecific Monoclonal Antibodies" - Principal Investigator

1992 American Cancer Society - Clinical Oncology Fellowship - Program Director

1993 Chiron Corporation - "Phase I Trials of 2B1 Bispecific Monoclonal Antibody" - Principal Investigator

1993 Chiron Corporation - "Anti-tumor Activity of Antigen Fork Bispecific Antibodies" - Principal Investigator

1993 Frank Strick Foundation - "Clinical Development of Bispecific Monoclonal Antibodies" - Principal Investigator

1994 Chiron Corporation - "Phase I Trials of 2B1 Bispecific Monoclonal Antibody" - Principal Investigator

1994 Pharmacia Corporation - "Phase I Trial of the Immunotoxin LS4565" - Principal Investigator

1995 Frank Strick Foundation - "Clinical Development of Novel Bispecific Monoclonal Antibodies" - Principal Investigator

1995 Pharmacia and Upjohn - "Phase I Trial of Repeated Doses of the Immunotoxin PNU214565" - Principal Investigator

1995 Bristol-Myers Squibb - "Phase I Trial of BR96-Doxorubicin" - Principal Investigator

1996 Frank Strick Foundation - "Immunotherapy of Cancer using Antibodies and their Engineered Derivatives" - Principal Investigator

1997 Pharmacia and Upjohn - "Adaptive dosing based on circulating anti-SEA antibodies using PNU214565" - Principal Investigator

1997 Frank Strick Foundation-"Development and Clinical Testing of Novel Immunotherapeutics". - Principal Investigator

1997 Smith-Kline Beecham – "Topotecan Clinical Trials" – Principal Investigator

1998 Janssen Pharmaceutica – "Pre-Targeted Radioimmunotherapy" – Principal Investigator

1998 Janssen Pharmaceutica – "Phase I trial of the Farnesyl transferase inhibitor R115777" – Principal Investigator

1998-2006 Frank Strick Foundation - "Immunotherapy of Cancer using Antibodies and their Engineered Derivatives" - Principal Investigator

1999 Abgenix Inc. – "An Open Label, Maintenance Dosing, Clinical Trial of ABX-EGF in Patients with Renal, Prostate, Pancreatic, Non-Small-Cell Lung, or Esophageal Cancer" – Principal Investigator

2001 Genentech – "Clinical Training Award" – Principal Investigator

2005 Eisai Corporation – "TLR4 Agonist Promotion of Antibody-Promoted Adaptive Immunity" – Principal Investigator

2006-08 Amgen Corporation – "High-Throughput siRNA Screening" – Co-Principal Investigator

MANUSCRIPT REVIEWER:

Cancer
Cancer Research
Clinical Cancer Research
Institute of Medicine Report – External Reviewer: "Shortening the Time Line for New Cancer Treatments"
Investigational New Drugs
Journal of Cellular Pharmacology
Journal of Clinical Investigation
Journal of Clinical Oncology
Journal of Immunotherapy
Lancet
Nature Biotechnology
New England Journal of Medicine
Oncology: International Journal of Cancer Research and Treatment
Science

GRANT REVIEWER:

Division of Research Grants, National Cancer Institute

1. Special Review Committees, March 1990, August 1990, April 1991, July 1991
2. ad hoc Reviewer, Small Business Innovation Research, July 1990
3. Member, Experimental Therapeutics-2 Study Section, October, 1991-June, 1995.
4. ad hoc Reviewer, Study Section/Special Emphasis Panel to review RFA AI-00-006 "Innovative Grants on Immune Tolerance"
5. Member, NIH Clinical Trials Subcommittee C, 2001 - 2005.
6. ad hoc Reviewer, Study Section/Cancer Immunopathology and Immunotherapy-October, 2003.
7. Member, Cancer Immunopathology and Immunotherapy Study Section, December, 2004-June, 2008.

Grants Review Branch, Division of Extramural Activities, National Cancer Institute - NCI Center Support Grant Review Committee, June 1998

Dutch Cancer Society Review Board, The Netherlands, 1998-1999

Co-Director, American Cancer Society Institutional Review Committee, Fox Chase Cancer Center

External Advisory Committee, TUFTS/New England Medical Center's Training Grant in Clinical Care Research.

Cancer Panel Review Member, Doris Duke Distinguished Clinical Scientist Award (DCSA), 2002.

Chairman, UCSF U54 CA90788 External Advisory Committee, 2003 – Present

Member, Israeli Cancer Research Foundation (ICRF) Scientific Review Panel, 2004 - Present

Committee Member, Biologics Panel, NIH/NCI RAID, 2005 – Present

Committee Member, NCI/Translational Research Working Group (TRWG) 2005 - 2007

THESIS: Weiner, L.M.: Adaptive Responses of Mitochondria to Alterations in Ambient Oxygen Tension. Honors Thesis, Department of Biology, University of Pennsylvania; accepted May, 1973.

SELECTED INVITED LECTURES:

1986

Cancer and Science VII - Fox Chase Cancer Center - April, 1986

The Estelle Laska Memorial Series

The American Cancer Society - Chester County Unit - April, 1986

Sixth Annual Cancer Symposium - Western Reserve Care System - Youngstown, Ohio - April, 1986

Schreiner Symposium on Colorectal Cancer - Seattle, WA - October, 1986

Toward 2000, Philadelphia, PA, October, 1986

1987

Medical Grand Rounds - Temple University - February, 1987

Medical Grand Rounds - Robert Wood Johnson Medical School, Camden, New Jersey - October, 1987

1988

Biomodulation of Cancer - Society for Biological Therapy, San Francisco, California - November, 1988

1989

Review of Medical Oncology - Montefiore Medical Center, New York, New York - March 1989

Monoclonal Antibody Conjugates - UCSD Cancer Center, San Diego, California - March 1989

Tumor Conference - Christiana Medical Center, Wilmington, Delaware - October 1989

1990

Grand Rounds - Lankenau Hospital, Philadelphia, Pennsylvania - March 1990

13th Annual Cancer Update - Marquette General Hospital, Marquette, Michigan - March 1990.

Pittsburgh Cancer Institute Annual Symposium - University of Pittsburgh, Pittsburgh, PA - March 1990.

Bispecific Antibody Targeting Tumor and FcγRIII, 2nd International Conference on Bispecific Antibodies and Targeted Cellular Cytotoxicity, Seillac, France - October 1990.

1991

Biotherapy of Cancer: A Symposium for Clinicians and Nurses. Hoag Memorial Hospital, Newport Beach, CA. - February, 1991

European School of Oncology - Israel Cancer Association. Shoresh, Israel - October, 1991

1992

Eastern Cooperative Oncology Group Scientific Retreat - February, 1992

Toward 2000 VIII, Fox Chase Cancer Center, Philadelphia, PA - October, 1992

1993

FASEB Summer Conference, Saxtons River, VT - August, 1993

Monoclonal Antibody Therapy of Cancer, Contemporary Issues in Hematology/Oncology,

Univ. of Florida Health Science Center, Jacksonville, FL - November, 1993

Fourth Annual International Conference on Antibody Engineering, Coronado, CA - December, 1993

1994

28th Annual Main Line Conference, Bryn Mawr, PA, April 1994

Antibody-Based Therapeutics, Washington, D.C., June, 1994

Bispecific Monoclonal Antibody Therapy - Washington, DC - June 1994

1995

Session Chair, 4th International Conference on Bispecific Monoclonal Antibody Therapy, Hawks Cay, FL - March, 1995

Session Chair, Tenth International Conference on Monoclonal Antibody Immunoconjugates for Cancer, San Diego, CA - March, 1995

Chairperson, Poster Discussion Session, Bispecific Antibodies, Monoclonal Antibodies and Immunoconjugation, American Association for Cancer Research Annual Meeting, Toronto, Canada - March, 1995

Targeted Cancer Therapy Using Engineered Antibodies, Cancer Biology Seminar, Arizona Cancer Center, Tucson, AZ - October, 1995

Tumor-Targeting Properties of Anti-c-erbB-2 sFv, Therapeutic Antibody Technology 95 Symposium, San Francisco, CA - October, 1995

Session Chair and Speaker, Bispecific Anti-Her2/Neu Antibody Strategies, 10th Annual Meeting of the Society for Biological Therapy, Williamsburg, VA - November, 1995

Engineered Antibodies for Cancer Therapy, University of Pennsylvania Cancer Center, Philadelphia, PA - December, 1995

Targeted Therapy Using Engineered Antibodies, Multidisciplinary Clinical Conference, The Johns Hopkins Oncology Center, Baltimore, MD - December, 1995

1996

Session Chair and Speaker, Exploring and Exploiting Antibody and Ig Superfamily Combining Sites, Keystone Symposia, Taos, NM, February, 1996

Targeted Therapy of Cancer Using Engineered Antibodies, Robert L. Krigel Memorial Lectureship, Lankenau Hospital, Wynnewood, PA - April, 1996

Targeted Therapy Using Monoclonal Antibodies, University of Iowa Hematology-Oncology Grand Rounds, Iowa City, IA - May, 1996

Monoclonal Antibody Therapy of Human Malignancies, The Second Annual Northern New Jersey Cancer Center Research Symposium, Experimental & Clinical Approaches in Oncology: Approaching the 21st Century, Teaneck, NJ - May, 1996

Systemic Therapy for Rectal Cancer, Carcinoma of the Rectum: An Update for Physicians. William Beaumont Hospital, Royal Oak, MI - October, 1996

Session Chair and Speaker, Modulating binding affinity to improve the tumor-targeting properties of monomeric single-chain Fv molecules. IBC's Seventh Annual International Conference on Antibody Engineering, Coronado, CA - December, 1996

1997

Phase I trial of escalating doses of taxol in combination with cisplatin, fluorouracil and 60 Gy radiation prior to esophagectomy, Poster Discussion Session, The Fox Chase Cancer Center and Free University Hospital Investigator's Workshop and Consensus Conference, Rio Grande, PR - March, 1997

1997 Program Committee and Session Chair, Immunobiology and Gene Therapy Session, American Society of Clinical Oncology (ASCO), Denver, CO - May, 1997

Superantigen-targeted therapy: Phase I trials of PNU-214565, a fusion protein composed of staphylococcal enterotoxin A (SEA) and C242 Antibody Fab fragment in patients with advanced gastrointestinal malignancies, Speaker and Session Chairperson, IBC Conference on Antibody-Based Therapeutics, Boston, MA - June, 1997

Antibody-targeted activation of cellular immunity. Speaker at Symposium on The future use of monoclonal antibodies in cancer therapy at ECCO 9 -The European Cancer Conference, Hamburg, Germany - September, 1997

Targeted cellular therapy using bifunctional proteins. Department of Biochemistry, University of Virginia, Charlottesville, VA - October, 1997

1998

Antibody-directed superantigen therapy. Speaker, 11th International Conference on Monoclonal Antibodies for Cancer, San Diego, CA – March, 1998

Future directions: Review and outlook of Paclitaxel in esophageal cancer. Speaker, The Fox Chase Cancer Center and Free University Hospital Investigator's Workshop and Consensus Conference, St. Thomas, VI - March, 1998

Engineering antibodies for cancer therapy. Speaker, Advances in the Applications of Monoclonal Antibodies in Clinical Oncology, Thira, Santorini, Greece – May, 1998

Engineered antibodies for cancer therapy. Speaker, NMHCC's Bio/Technology Division's Cancer Immunotherapy & Gene Therapy Conference, Arlington, VA – June, 1998

The development of monoclonal antibodies in medical oncology. Speaker, MD Anderson Cancer Center Medical Oncologist Consensus Conference on Future Directions with Herceptin for Breast Cancer, Wailea, Maui, HA – July, 1998

Faculty, ASCO/AACR Workshop on Methods in Clinical Cancer Research, Vail, CO – July, 1998

Breast cancer therapies. 13th annual Meeting of Society for Biological Therapy, University of Pittsburgh, Pittsburgh, PA – October, 1998

Biological therapy of cancer. 23rd Annual Michael Wohl Memorial Lecture, Temple University School of Medicine, Philadelphia, PA – November, 1998

Cancer Therapy using Engineered Antibodies. Speaker and Chairperson, IBC Ninth Annual International Conference on Antibody Engineering, Coronado, CA – December, 1998

1999

Clinical Strategies with Monoclonal Antibody Therapy, Speaker, Robert H. Lurie Comprehensive Cancer Center Oncology Consensus Conference on Recent Advances and Future Directions using Monoclonal Antibodies for B-Cell Malignancies, Kona, HA – January, 1999

Antibody therapy. Visiting Professor and lecturer, Course: "Viruses, Cancer, and Immunology", Dept. of Biological Sciences, Lehigh University, Bethlehem, PA – February, 1999

Manipulation of antibody/effector cell interactions. Participant, Monoclonal Antibodies in Oncology meeting, Genzyme Transgenics Corp., Framingham, MA – February, 1999

Monoclonal antibody therapy of cancer. 11th Annual Cancer Progress Conference, New York, NY – March, 1999

Therapeutic monoclonal antibodies for malignant disease. American Federation for Medical Research Symposium, Washington, DC – April, 1999

Novel Approaches in Pancreas Cancer Treatment. Marc Lustgarten Foundation for Pancreas Cancer Research, New York, NY – April, 1999

Targeted Cellular Cytotoxicity: The VIth Int'l Conference in Bispecific Antibodies and Related Strategies for Targeted Immune Modulation, Conference Co-Chair and speaker, Pacific Grove, CA – July, 1999

Faculty, ASCO/AACR Workshop on Methods in Clinical Cancer Research, Vail, CO – August, 1999

Bispecific Antibodies in Cancer Therapy. 1999 Oncology Frontiers Conference, Advisor and speaker, St. Thomas, US Virgin Islands - October, 1999

2000

Antibody-based fusion proteins as platforms for cancer therapy. Gordon Research Conference on Drug Carriers in Medicine & Biology, Ventura, CA - February, 2000

Immunotherapy with Cytokines and Naked Antibodies. International Conference on Advances in Cancer Immunotherapy: Organizing Committee, Session Chair and Speaker, Princeton, NJ - March, 2000

The Biological Therapy of Cancer. Grand Rounds, Graduate Hospital Department of Medicine, Philadelphia, PA – March, 2000

Predictors of Therapeutic Response/Discussion Session. Co-Chairperson Poster Discussion Session. American Association for Cancer Research annual meeting, San Francisco, CA – April, 2000.

Fellow Series: How to Write a Grant. Special Session presentation at annual American Society of Clinical Oncology, New Orleans, LA – May, 2000.

Treatment of epithelial tumors with monoclonal antibodies. Monoclonal Antibodies for the Treatment of Malignant Diseases: Present Achievements and Future Prospects. Speaker; Stockholm, Sweden – April, 2000

Biological therapy of cancer. Kitty Cookson Memorial Lecture. Royal Free and University College, London, England – July, 2000

Faculty, ASCO/AACR Workshop on Methods in Clinical Cancer Research, Vail, CO – August, 2000

Developmental Therapeutics. Keynote Speaker, Beth Israel-Deaconess Medical Center Annual Cancer Center Retreat, Woods Hole, MA – September, 2000

Treatment of Breast Cancer and Other Tumors with Monoclonal Antibodies. 59th Japanese Cancer Association, Tokyo, Japan – October, 2000

Cancer Therapy Using Engineered Antibodies. DuPont Pharma Seminar Series. Wilmington, DE – October, 2000.

Clinical and PreClinical Advances with Engineered Antibodies. Session Chair, IBC 11th Int'l Conference on Antibody Engineering, La Jolla, CA – December, 2000

Translation of Fundamental Advances in Immunology to Clinical Practice. Speaker and Chairperson, Scientific and Technological Innovations in Biology: Initiating Advances in Therapeutic Approaches to Hematologic Malignancies, American Society of Hematology, San Francisco, CA – December, 2000

2001

Novel Approaches to the Immunotherapy of Colorectal Cancer and Other Solid Tumors. Keystone Symposium on Molecular Medicine of Colorectal Cancer, Taos, NM – February, 2001.

Faculty, National Medical Oncology Fellows Forum. Orlando, FL – March, 2001.

Current Status of Antibodies in Cancer Therapy. 54th Annual Society of Surgical Oncology Symposium, Washington, DC – March, 2001.

Engineered Antibodies for Cancer Therapy. US-Japan Workshop on Recent Advances in Specific Immunotherapy of Cancer, Maui, HA – March, 2001.

Manipulation of the tumour microenvironment to facilitate antibody therapy. Speaker and Session Chairperson, VIIth International Antibody Conference on Targeted Cellular Cytotoxicity, Hampshire, UK – August, 2001.

Protein Engineering to Optimize Affinity and Efficacy of Monoclonal Antibodies. Congress on Monoclonal Antibodies, Banff, Canada – September, 2001.

ABX-EGF Receptor Antibody: Current Status and Future Directions. Congress on Monoclonal Antibodies, Banff, Alberta, Canada – September, 2001.

Antibody-based therapy of cancer. Research Grand Rounds, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL – November, 2001.

Manipulation of the tumor micro-environment to facilitate antibody therapy. IBC 12th Int'l Conference on Antibody Engineering, San Diego, CA – December, 2001.

2002

Monoclonal Antibodies. 43rd Annual Clinical Conference – Drug Discovery and Clinical Evaluation in the 21st Century, Houston, TX – January, 2002.

Antibody therapy of cancer – New concepts and approaches. Clinical and Translational Seminar Series, Cardinal Bernardin Cancer Center, Chicago, IL – February, 2002.

Tumor targeting by engineered antibodies. Interdisciplinary seminar series of the Institute for Medicine and Engineering (IME) of the University of Pennsylvania, Philadelphia, PA – March, 2002.

Discussant, Biologic and Targeted Therapies Oral Presentation Session. ASCO, Orlando, FL – May, 2002.

New antibody structures as therapeutic delivery vehicles. Planning Committee, Session Moderator and Speaker, 2nd Int'l Congress on Monoclonal Antibodies in Cancer, Banff, Alberta, Canada – August, 2002.

Protein engineering to optimize affinity of monoclonal antibodies. First Annual Symposium on Anti-Receptor Signaling in Human Neoplasia, Chicago, IL – September, 2002.

Engineered antibodies for cancer therapy. Excellence in Immunology Lecture Series, University of Texas Southwestern Medical Center at Dallas, Dallas, TX – December, 2002.

2003

Keystone Symposium: Antibody-Based Therapeutics for Cancer. Program Chairperson and Speaker, Banff, Alberta, Canada – February, 2003.

New approaches in monoclonal antibodies for cancer. XIV Cancer Progress Conference, New York, NY – March, 2003.

EGFR Antibodies: Are they different from the TK inhibitors? Speaker and Program Committee, 4th International Lung Cancer Congress, Maui, HA – June, 2003.

Faculty, ASCO/AACR Methods in Clinical Research Workshop, Vail, CO – July, 2003.

Immunology Program Chair and Speaker, 94th Annual Meeting of the American Association for Cancer Research, Washington, DC – July, 2003.

ABX-EGF: A high-affinity, fully human antibody to EGFR. Speaker, Session Moderator and Planning Committee Member, Third Int'l Congress on Monoclonal Antibodies in Cancer, Quebec, Canada – August, 2003.

Engineered antibodies for cancer therapy. Speaker, Int'l Society for Oncodevelopmental Biology and Medicine (ISOBM) 2003 Symposium, Edinburgh, UK – September, 2003.

Antibody therapy for the treatment of cancer. Speaker, 7th Int'l Meeting and 1st World Congress, Biotherapy of Cancer: From Disease to Targeted Treatment, Munich, Germany – September, 2003.

Monoclonal antibody therapy of cancer: Targeted therapy hits the target. Vermont Cancer Center Grand Rounds, University of Vermont, Burlington, VT – September, 2003.

Monoclonal antibodies in cancer therapy. Speaker, Cancer Education Consortium/ Genentech/IDEC Clinical Pharmacology of Anticancer Agents, Leesburg, VA – October, 2003.

Monoclonal antibody therapy - What's Next? Visiting Professor, EMD Pharmaceuticals, Inc., Durham, NC – October, 2003.

Approaches to the future treatment of solid tumors and metastatic disease in cancer. Speaker, Session Chair, and Scientific Advisory Board for IBC's 14th Int'l Antibody Engineering Conference, San Diego, CA – December, 2003.

Monoclonal antibody therapy: New targets for magic bullets. Division of Cancer Medicine Grand Rounds speaker, M.D. Anderson Cancer Center, Houston, TX – December, 2003.

2004

Program Co-Chair, Immunology Program Chair, Symposium Co-Chair and Sunrise Session Speaker, 95th Annual Meeting of the American Association for Cancer Research, Orlando, FL – March, 2004.

Monoclonal antibody therapy-What' Next? Session Leader, Master of Excellence in Medicine-Oncology Option, Institut Superieur de Formation A L'Excellence en Medecine, Videoconference to Paris, France – March, 2004.

Developmental Therapeutics: Immunotherapy Poster Session Discussant, 2004 Annual Meeting of American Society of Clinical Oncology, New Orleans, LA – June, 2004.

The Contributions of Nuclear Medicine in the Practice of Medical Oncology: Expectations of Medical Oncology. Society of Nuclear Medicine 51st Annual Meeting, Philadelphia, PA – June, 2004.

Faculty, ASCO/AACR Methods in Clinical Research Workshop, Vail, CO – August, 2004.

Evolving treatment options in colorectal cancer. Update on GI Neoplasia Conference, Philadelphia, PA – August, 2004.

Panitumumab (ABX-EGF): A high affinity, fully human antibody to EGFR. Speaker and Session Moderator, 4th International Congress on Monoclonal Antibodies Workshop, Colorado Springs, CO – September, 2004.

Preclinical and clinical development of recombinant antibodies. Session Chair and Scientific Advisory Board for IBC's 15th Int'l Antibody Engineering Conference, San Diego, CA – December, 2004.

2005

Antibody-based therapeutics for cancer: Keystone Symposia Program Co-Chair and Speaker, Santa Fe, NM – February, 2005.

The next generation in monoclonals - Who's on first? Sixteenth Annual Cancer Progress Conference, New York, NY – March, 2005.

Immunology and Immunotherapy: Special Interest Session. Scientist - Survivor Program, American Association for Cancer Research Annual Meeting, Anaheim, CA – April, 2005.

Chairperson, Targeted Strategies in Cancer Therapy: Improving Patient Outcomes, 2005 ASCO Satellite Symposium, Orlando, FL – May, 2005.

Presenter, Molecular Therapeutics Poster Session, and Discussant, Developmental Therapeutics: Immunotherapy Poster Session, 2005 Annual Meeting of American Society of Clinical Oncology, Orlando, FL – May, 2005.

Co-Chair Symposia Session #34, Antibodies: Innate versus Engineered, Era of Hope 2005/DOD Breast Cancer Research Program, Philadelphia, PA – June, 2005.

Faculty, ASCO/AACR Methods in Clinical Research Workshop, Vail, CO – August, 2005.

Panitumumab in Colorectal Cancer. Speaker and Session Moderator and Program Planner, 5th International Congress on Monoclonal Antibodies in Cancer, Quebec City, Canada – August, 2005.

New Directions in Cancer Treatment. Carcinoid/NET 2005 Conference, Philadelphia, PA – September, 2005.

Antibodies as the "Drug" for Cancer Therapy. Keynote speaker, Monoclonal Antibodies in Cancer, Philadelphia, PA – September, 2005.

Manipulating Antibody Affinity for Cancer Therapy: Too Much of a Good Thing? Speaker, Yale Cancer Center Grand Rounds, New Haven, CT – September, 2005.

Engineered Antibodies for Cancer Therapy. Speaker, Lankenau Hospital Fall Seminar Series, Wynnewood, PA – October, 2005.

Customizing Antibody Affinities for Cancer Therapy. Session Chair and Program Planner for IBC's 16th Int'l Antibody Engineering Conference, San Diego, CA – December, 2005.

2006

Current Clinical Approaches of Targeting EGFR in Colorectal Cancer. Faculty, Optimizing EGFR-Targeted Therapies in Colorectal Cancer Symposium, San Francisco, CA – January, 2006.

Engineered Antibodies for Cancer Therapy. Speaker, Carole and Ray Neag Comprehensive Seminar Series, University of Connecticut Health Center, Farmington, CT – February, 2006.

Customizing Antibody Affinities for Cancer Therapy. Sidney Kimmel Cancer Center Conference on Proteogenomics for Diagnosis, Imaging and Therapy of Cancer, San Diego, CA – February, 2006.

Immunology and Immunotherapy: Special Interest Session. Scientist - Survivor Program, 97th Annual American Association for Cancer Research, Washington, DC – April, 2006.

Customizing Antibodies for Cancer Therapy. Sunrise Session, 97th Annual Meeting of American Association for Cancer Research, Washington, DC – April, 2006.

Update on the Management of Colon Cancer. Lawrence M. Sigman, M.D. Memorial Lecture, Jeanes Hospital Department of Medicine; Philadelphia, PA - April, 2006

Frankly speaking about new discoveries in cancer: Special focus on colorectal cancer. 8th Annual Celebration of Hope; The Wellness Community of Philadelphia, Philadelphia, PA – June, 2006.

Faculty, FECS/AACR/ASCO Methods in Clinical Cancer Research, Flims, Switzerland – June, 2006.

Faculty, ASCO/AACR Methods in Clinical Research Workshop, Vail, CO – July, 2006.

Program Committee, Session Chair and Moderator and Speaker, 6th Int'l Congress on Monoclonal Antibodies in Cancer Symposium, Washington, DC – August, 2006.

Novel EGFR Inhibitors. Faculty, Current Trends in GI Malignancies Meeting, Philadelphia, PA – September, 2006.

Engineering antibodies for cancer therapy. Department of Immunology Grand Rounds, Roswell Park Cancer Institute, Buffalo, NY – September, 2006.

Engineering antibodies for cancer immunotherapy. UMDNJ/Cancer Institute of New Jersey Grand Rounds, New Brunswick, NJ – October, 2006.

Unconjugated antibodies for cancer therapy. Session Moderator and Speaker, 11th Conference on Cancer Therapy with Antibodies and Immunoconjugates, Parsippany, NJ – October, 2006.

Co-Chair, Mini-Symposium on Biologic Effects of Targeted Therapeutics and Session Co-Chair, 21st Annual Meeting of the Int'l Society for Biological Therapy of Cancer, Los Angeles, CA – October, 2006.

Monoclonal antibodies for cancer immunotherapy. Abramson Family Cancer Research Institute/Division of Hematology-Oncology at the University of Pennsylvania, Philadelphia, PA – November, 2006.

Session Chair, Immunoprevention. AACR Int'l Conference on Frontiers in Cancer Prevention Research, Boston, MA – November, 2006.

Conference Co-Chair, Tumor Immunology: An Integrated Perspective; AACR Special Conference in Cancer Research, Miami, FL – November, 2006.

Improving anti-tumor antibody-initiated ADCC. Faculty, IBC's 17th Annual Int'l Conference on Antibody Engineering: Antibody Engineering and Immunotherapeutics for the 21st Century, San Diego, CA – December, 2006.

2007

Cancer therapy using unconjugated monoclonal antibodies: Emerging concepts. LSU-Tulane Health Sciences Cancer Center Seminar Series, New Orleans, LA – January, 2007.

Engineered antibodies for cancer therapy. Drexel University School of Biomedical Engineering Seminar, Philadelphia, PA – February, 2007.

Promoting ADCC by natural killer cells. Keystone Symposium, Lake Louise, Alberta – February, 2007.

Obstacles to implementing cancer vaccines. NCI/FDA Workshop on Rapid Translation of Research Findings into Clinical Practice, Bethesda, MD – February, 2007.

Frankly speaking about new discoveries in cancer. The Wellness Community of Philadelphia, Philadelphia, PA – March, 2007.

New directions for monoclonal antibody therapy of cancer. Distinguished Scientists Seminar, Centocor Corp., Malvern, PA – March, 2007.

Engineered antibodies for cancer therapy. Sidney Kimmel Cancer Center of Johns Hopkins University, Baltimore, MD – March, 2007.

Session Chair, Educational Session "Toll Receptors and Cancer" at Annual Meeting of American Association of Cancer Research, Los Angeles, CA – April, 2007.

Engineering antibodies for cancer therapy. Keynote Speaker, The Future of Monoclonal Antibody Biotherapeutics Production and Development Symposium, New York Academy of Sciences, New York, NY – May, 2007.

MEMBERSHIPS/ADVISORY BOARDS:

National Committees

Eastern Cooperative Oncology Group (ECOG)
- Principal Investigator, Fox Chase Cancer Center (1996-2002)
- Chairman, Biologic Response Modifiers Committee (1996-2002)
- Member, Gastrointestinal Cancer Committee
- Member, Laboratory Science Committee
- Member, ECOG Board of Advisors
- Member, Audit Committee

Member, PDQ External Advisory Board - NCI
Member, PDQ Adult Treatment Editorial Board - NCI
American Association for the Advancement of Science (AAAS)
American Association for Cancer Research (AACR)
- Chairperson, Cancer Immunology Task Force
- Incoming Course Director, AACR/ASCO Clinical Methodology Workshop
- AACR Foundation Steering Committee
- Translational Research Committee
- Chair, Tumor Immunology Special Conference, November 2006
- Education Committee, 2007 Annual Meeting
- AACR Special Conferences Committee 2007-2010

American Society of Clinical Oncology (ASCO)
American Society of Hematology (ASH)
American Society of Immunology (ASI)
American Federation for Clinical Research (AFCR)
American Federation for Medical Research (AFMR)
Clinical Immunology Society (CIS)
Pennsylvania Oncologic Society (POS)
Society for Biological Therapy (SBT)
The Antibody Society (TabS)
External Advisory Board, Tufts-NEMC Cancer Center (2005 – present)
NCI RAID Program Review – September, 2005
NCI Translational Research Working Group (TRWG) (2005 – present)
- Parent Committee
- Prioritization Subcommittee

Other

Member, PROGRESS Editorial Advisory Board
Scientific Advisory Board, Immunotherapy Corporation (1998 – 2000)
Scientific Advisory Board, Cell Pathways Corporation (1998 – 2002)
Scientific Advisory Board, Millennium Pharm. (1999 – 2001)
Medical Advisory Board, Abgenix, Inc. (1998 – 2006)
Scientific Advisory Board, Celldex Therapeutics, Inc. (2005 – present)
Cancer Advisory Board, Serono Research Institute (2004 – present)
Hematology-Oncology Editorial Advisory Board, Medscape, LLC (2005-present)
Scientific Advisory Board, Merrimack Pharmaceuticals (2006 – present)
International Oncology Advisory Board, Johnson and Johnson (2006 – present)

ADMINISTRATIVE:

Member, FCCC Medical Science Division Appointments & Promotions Committee, 1992 - 1998.
Member, FCCC Intensive Care Unit Committee, 1986 - 1992.
Member, FCCC Laboratory Animal Committee, 1989 - 1994.
Director, FCCC Medical Oncology Fellowship Program, 1986 - 1994.
Director, FCCC Clinical Investigator Training Program, 1993 - Current.
Member, FCCC Executive Committee of Staff, 1994 - .
Member, FCCC Ambulatory Care Working Group Committee, 1994 - 2004
Co-Director, American Cancer Society Institutional Grant Review Board, 1995 - Present.
Member, FCCC Centerwide Appointments & Promotions Committee, 1999 - 2005
Member, Faculty Advisory Committee for Institutional Advancement, 2003 - Present.
Member, Internal Advisory Board of the Medical Outreach and Minority Affairs Program, 2005 - Present.
Member, Fox Chase/Temple Affiliation Executive Committee, 2005 – Present.
Member, FCCC Extramural Research Leadership Committee, 2006 – Present.
Member, FCCC Strategic Vision Committee 2007-
Member, FCCC Translational Research Committee 2007-

BIBLIOGRAPHY:

PUBLISHED ARTICLES, REVIEWS, CHAPTERS:

1. Wilson DF, Owen C, Mela L, **Weiner LM**. Control of Mitochondrial Respiration by the Phosphate Potential Biochemica and Biophysica Acta. 53:326-33, 1973.

2. Nordheim A, Pardue ML, **Weiner LM,** Lowenhaupt K, Scholten P, Moller A, Rich A, Stollar BD. Analysis of Z-DNA in Fixed Polytene Chromosomes with Monoclonal Antibodies that Show Base Sequence-dependent Selectivity in Reactions with Supercoiled Plasmids and Polynucleotides. J Biological Chem.Vol 261 (1):468-476, 1986.

3. **Weiner LM**, Steplewski Z, Koprowski H, Sears HF, Litwin S, Comis RL. Biologic Effects of Gamma Interferon Pre-Treatment Followed by Monoclonal Antibody 17-1A Administration in Patients with Gastrointestinal Carcinoma. Hybridoma. 5 (Suppl. 1): 65-77, 1986.

4. Paul AR, Engstrom PF, **Weiner LM**, Steplewski Z, Koprowski H. Treatment of Advanced Measurable or Evaluable Pancreatic Carcinoma with 17-1A Murine Monoclonal Antibody Alone or in Combination with 5-Fluorouracil, Adriamycin and Mitomycin (FAM) Hybridoma. 5 (Suppl.1): 171-174, 1986.

5. Schwob VS, **Weiner LM**, Hudes G, Ratech H. Extranodal Non-T cell Lymphoblastic Lymphoma in Adults: A New Clinicopathologic Entity. Human Pathology. Am J Clin Path. 90:602-605, 1988.

6. **Weiner LM**. Monoclonal Antibody Therapy. AAOHN Journal. 35:4, 1987.

7. **Weiner LM**, Steplewski Z, Koprowski H, Litwin S, Comis RL. Divergent Dose-Related Effects of Interferon-γ Therapy on In Vitro Antibody-Dependent Cellular and Non-Specific Cytotoxicity by Human Peripheral Blood Monocytes. Cancer Res. 48:1042-1046, 1988.

8. **Weiner LM**, Moldofsky P, Gatenby R, O'Dwyer J, O'Brien J, Litwin S, Comis R. Antibody Delivery and Effector Cell Activation in a Phase II Trial of Recombinant Interferon-Gamma and the Murine Monoclonal Antibody CO17-1A in Advanced Colorectal Carcinoma. Cancer Res. 48:2568-2573, 1988.

9. **Weiner LM**. Biologic Response Modifiers in Cancer Treatment. Welcome Trends in Hospital Pharmacy. 10:7-12, 1988.

10. Gatenby RA, Moldofsky PJ, **Weiner LM**. Correlation of Tumor Oxygen Levels and Uptake of Radiolabeled $F(ab')_2$ Monoclonal Antibody Fragments in Metastatic Colon Carcinoma Radiology. 166:757-759, 1988.

11. **Weiner LM**, Zarou C, O'Brien J, Ring D. Effector Characteristics of the IgG_3 Murine Monoclonal Antibody 113F1. J Biol Resp Mod. 8:227-237, 1989.

12. **Weiner LM**, O'Dwyer J, Kitson J, Comis RL, Frankel AE, Bauer RJ, Konrad, MS, ES Groves. A Phase I Evaluation of an Anti-Breast Carcinoma Monoclonal Antibody 260F9-Recombinant Ricin A chain immunoconjugate. Cancer Res. 49:4062-4067, 1989.

13. Gould BJ, Borowitz MJ, Groves ESD, Carter PW, Anthony D, **Weiner LM**, Frankel AE. A Phase I Study of an Anti-Breast Cancer Immunotoxin by Continuous Infusion: Report of a Targeted Toxicity Not Predicted by Animal Studies. JNCI. 81:775-781, 1989.

14. Heda GD, Mardente S, **Weiner LM**, Schmaier AH. Interferon Gamma increases in vitro and in vivo Expression of C1 Inhibitor. Blood. 75:2401-2407, 1990.

15. O'Dwyer PJ, Paul AR, Walczak J, **Weiner LM**, Litwin S, Comis RL. Phase II Study of Biochemical Modulation of 5-Fluorouracil by Low-Dose PALA in Patients with Colorectal Cancer. J Clin Onc. 8:1497-1503, 1990.

16. Garcia Palazzo IE, Gercel-Taylor C, Kitson J, **Weiner LM**. Potentiation of Tumor Lysis by a Bispecific Antibody That Binds to CA19-9 Antigen and the Fcγ Receptor Expressed by Human Large Granular Lymphocytes. Cancer Res. 50:7123-7128, 1990.

17. **Weiner LM**, dePalazzo IG, Kitson J, Gercel-Taylor C. Biologic Properties of a Bispecific Monoclonal Antibody Directed Against CA19-9 Antigen and FcγRIII. Proceedings: 2nd International Conference on Bispecific Antibodies and Targeted Cellular Cytotoxicity. Seillac, France, 1990.

18. Perez RP, Padavic K, Krigel R, **Weiner LM**. Anti-Erythrocyte Autoantibody Formation Following Therapy with Interleukin-2 and Gamma Interferon. Cancer. 67:2512-2517, 1991.

19. Krigel RL, Padavic-Shaller KA, Rudolph AR, Young JD, **Weiner LM**, Konrad M, Comis RL. Hemorrhagic Gastritis as a New Dose-Limiting Toxicity of Recombinant Tumor Necrosis Factor. J Natl Cancer Inst. 83:129-131, 1991.

20. **Weiner LM**, Padavic-Shaller K, Kitson J, Watts P, Krigel RL, Litwin S. Phase I Evaluation of Combination Therapy with Interleukin-2 and Gamma-interferon. Cancer Res. 51:3910-3918, 1991.

21. **Weiner LM**. Applications of Gamma-Interferon in Cancer Therapy. Molecular Biotherapy. 3:186-191, 1991.

22. dePalazzo IG, Kitson J, Gercel-Taylor C, Adams S, **Weiner LM**. Bispecific Monoclonal Antibody Regulation of FcγRIII-Directed Tumor Cytotoxicity by Large Granular Lymphocytes. Cell Immunol. 142:338-347, 1992.

23. Garcia de Palazzo IE, Holmes M, Alpaugh K, **Weiner LM**. Use of the Tumor Spheroid Model in Immunotherapy, in Tumor Immunobiology; A Practical Approach, eds, G. Gallagher, R. C. Rees, C. W. Reynolds, IRL Press at Oxford University Press, London, pp.385-397, 1993.

24. **Weiner LM**, Hudes GR, Kitson J, Walczak J, Watts P, Litwin S, O'Dwyer PO. Preservation of Immune Effector Cell Function Following Administration of a Dose-Intense 5-FU Based Chemotherapy Regimen. Cancer Immunol Immunother. 36:185-190, 1993.

25. Garcia de Palazzo IE, Holmes M, Gercel-Taylor C, **Weiner LM**. Antitumor Effects of a Bispecific Antibody Targeting CA19-9 Antigen and CD16. Cancer Res. 52:5713-5719, 1992.

26. **Weiner LM**, Harvey E, Padavic-Shaller K, Willson JKV, Walsh C, LaCreta F, Khazaeli MB, Kirkwood JM, Haller DG. Phase II Multicenter Evaluation of Prolonged Murine Monoclonal Antibody 17-1A Therapy in Pancreatic Carcinoma. J Immunother. 13:110-116, 1993.

27. **Weiner LM**, Holmes M, Adams GP, LaCreta F, Watts P, de Palazzo IG. A Human Tumor Xenograft Model of Therapy with a Bispecific Monoclonal Antibody Targeting c-erbB-2 and CD16. Cancer Res. 53:94-100, 1993.

28. **Weiner LM**, Holmes M, Richeson A, Godwin A, Adams GP, Hsieh-Ma ST, Ring DB and Alpaugh RK. Binding and Cytotoxicity Characteristics of the Bispecific Murine Monoclonal Antibody 2B1. J Immunology 151:1-9, 1993.

29. Garcia de Palazzo IE, Klein-Szanto A, **Weiner LM**. Immunohistochemical Detection of C-erbB2 expression by neoplastic human tissue using monospecific and bispecific monoclonal antibodies. Int. J Biomarkers 8:233-239, 1993.

30. McCartney JE, Tai MS, Opperman H, Jin D, Warren FD, **Weiner LM**, Bookman MA, Stafford III, WF, Houston LL, Huston JS. Refolding of single-chain Fv with C-terminal cysteine (sFv'): Formation of disulfide-bonded homodimers of anti-c-erbB-2 and anti-digoxin sFv'. Miami Short Reports 3:91, 1993.

31. Garcia de Palazzo IE, Adams GP, Sundareshan P, Wong AJ, Testa JR, Bigner DD, **Weiner LM**. Expression of mutated epidermal growth factor receptor by non-small cell lung carcinomas Cancer Res. 53:3217-3220, 1993.

32. Adams GP, McCartney JE, Tai M-S, Oppermann H, Huston JS, Stafford III WF, Bookman MA, Fand I, Houston LL, **Weiner LM**. Highly specific in vivo tumor targeting by monovalent and divalent forms of 741F8 anti-c-erbB-2 single-chain Fv. Cancer Res. 53:4026-4034, 1993.

33. Shpitz B, Chambers CA, Singhal AB, Hozumi N, Fernandes BJ, Roifman CM, **Weiner LM**, Roder JC, Gallinger S. High level functional engraftment of severe combined immunodeficient mice with human peripheral blood lymphocytes following pretreatment with radiation and anti-asialo GM_1. J Immunol Methods. 169:1-15, 1994.

34. **Weiner LM**, Li W, Holmes M, Catalano RB, Dovnarsky M, Padavic K, Alpaugh RK. Phase I trial of recombinant macrophage colony-stimulating factor and recombinant gamma-interferon: Toxicity, monocytosis, and clinical effects. Cancer Res. 54:4084-4090, 1994.

35. Huston JS, Adams GP, McCartney JE, Tai M-S, Oppermann H, Liu S, Stafford III WF, Bookman MA, Fand I, Apell J, Laminet AA, Houston LL, **Weiner LM**. Tumor targeting in a tumor xenograft model with the $(sFv')_2$ divalent forms of anti-c-erbB-2 single chain Fv. Cell Biophysics. 24/25:249-257, 1994.

36. Clark JI, **Weiner LM**. Monoclonal antibodies in cancer treatment. Resident and Staff Physician. 40:13-20, 1994.

37. Coia L, Hoffman J, Scher, R, Weese J, Solin L, **Weiner LM**, Eisenberg B, Paul A, Hanks G. Preoperative chemoradiation for adenocarcinoma of the pancreas and duodenum. Int J Rad Onc Bio Phys. 30:161-167, 1994.

38. Redei I, Green F, Hoffman JP, **Weiner LM**, Scher R, O'Dwyer PJ. Phase II trial of PALA and 6-methylmercaptopurine riboside (MMPR) in combination with 5-fluorouracil in advanced pancreatic cancer. Invest New Drugs. 12:319-321, 1994.

39. McCartney JE, Tai M-S, Hudziak RM, Adams GP,**Weiner LM**, Jin D, Stafford III WF, Liu S, Bookman MA, Laminet AA, Fand I, Houston LL, Opperman H, Huston JS. Engineering disulfide-linked single-chain Fv dimers $[(sFv')_2]$ with improved solution and targeting properties. Anti-digoxin 26-10 $(sFv')_2$ and anti-c-erbB2 741F8 $(sFv')_2$ made by protein folding and bonded through carboxyl-terminal cysteine peptides. Protein Engineering. 8:301-314, 1994.

40. **Weiner LM**, Houston LL, Huston JS, McCartney JE, Laminet AA, Apell G, Stafford WF, III, Bookman MA, Gallo J, Adams GP. Effects of continuous infusion on the biodistribution of the divalent anti-c-erb-2 single-chain Fv molecule, 741F8-1 $(sFv')_2$. Tumor Targeting. 1:51-60, 1995.

41. Langer CJ, Catalano R, **Weiner LM**, Scher R, Bagchi P, Saren B, Comis RL. Phase II evaluation of methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC) in advanced, measurable breast carcinoma. Cancer Investigation. 13(2):150-159, 1995.

42. Eisenberg BL, Szarka C, Frucht H, Lanciano RM, Hageboutros A, **Weiner LM**. Gastric Cancer In Cancer Surgery, eds. Harvey JC and Beattie EJ, W. B. Saunders Co, Philadelphia, pp. 53-76, 1995.

43. **Weiner LM**, McCartney JE, Tai M-S, Oppermann H, Huston JS, Liu S, Stafford III WF, Bookman MA, Fand I, Apell J, Laminet A., Houston LL, Adams GP. Specific in vivo tumor targeting by monovalent and divalent forms of the anti-c-erbB-2 single chain Fv molecule 741F8. Fourth Annual Intl Conf on Antibody Engineering. Coronado, CA.

44. Algan O, Coia LR, Keller SM, Engstrom PF, **Weiner LM**, Schultheiss TE, Hanks GE. Management of adenocarcinoma of the esophagus with chemoradiation alone or chemoradiation followed by esophagectomy. Results of sequential non-randomized Phase II studies. .J Rad Onc Biol Phys. 32:753-761, 1995.

45. Adams GP, McCartney JE, Wolf EJ, Oppermann H, Tai M-S, Eisenberg J, Giantonio B, Schultz J, Bookman MA, Laminet A, Apell G, Stafford III WF, Huston JS, Houston LL, **Weiner LM**. 741F8 sFv, an anti-c-erbB-2 single-chain Fv molecule: Preclinical studies and optimization of specific tumor delivery. Intl J Pharmacognosy. 33(S):75-91, 1995.

46. Clark JI, Alpaugh RK, **Weiner LM**. Natural killer cell-directed bispecific antibodies, In Molecular Biology Intelligence Unit: Bispecific Antibodies, ed Fanger, MW, R.G. Landes Co, Austin, pp. 77-87, 1995.

47. Adams GP, McCartney JE, Wolf EJ, Eisenberg J, Tai M-S, Huston JS, Stafford III WF, Bookman MA, Houston LL, **Weiner LM**. Optimization of in vivo tumor targeting with divalent forms of 741F8 single-chain Fv: Effects of dose escalation and repeated i.v. administration. Cancer Immunol and Immunother. 40:299-306, 1995.

48. Krigel RL, Padavic-Shaller K, Toomey, Comis RL, **Weiner LM**. A Phase I study of sequentially administered rTNF and rIL2. J Immunother Emphasis Tumor Immunol. 17:161-170, 1995.

49. Saleh MN, Goldman SJ, LoBuglio AF, Beall AC, Sabio H, McCord MC, Minasian L, Alpaugh RK, **Weiner LM**, Munn DH. $CD16^{+}$ monocytes in patients with cancer: Spontaneous elevation and pharmacologic induction by recombinant human macrophage colony-stimulating factor. Blood. 85:2910-2917, 1995.

50. Tai MS, McCartney JE, Adams GP, Jin D, Hudziak RM, Oppermann H, Laminet AA, Bookman MA, Wolf EJ, Liu S, Stafford III WF, Fand I, Houston LL, **Weiner LM**, Huston JS: Targeting c-erbB-2 expressing tumors using single-chain Fv monomers and dimers[1]. Cancer Res. 55:5983s-5989s, 1995.

51. Schier R, Marks JD, Wolf EJ, Apell G, Wong C, McCartney JE, Bookman MA, Huston JS, Houston LL, **Weiner LM**, Adams GP. In vitro and in vivo characterization of a human anti-c-erbB-2 single-chain Fv isolated from a filamentous phage antibody library. Immunotechnology. 1:73-81, 1995.

52. Adams G, McCartney J, Wolf E, Eisenberg J, Huston J, Bookman M, Moldofsky P, Stafford W, Houston L, **Weiner LM**. Enhanced tumor specificity of 741F8-1 (sFv')2, an anti-c-erbB-2 single-chain Fv dimer mediated by a stable radioiodine conjugation. J Nuclear Med. 36:2276-2281, 1995.

53. George AJT, Jamar F, Tai M-S, Heelan BT, Adams GP, McCartney JE, Houston LL, **Weiner LM**, Oppermann H, Peters AM, Huston JS. Radiometal labeling of recombinant proteins by a genetically engineered, minimal chelation site: coordination of technetium-99m by single-chain Fv antibody fusion protein through a carboxcyl-terminal cysteinyl peptide. Proc Natl Acad Sci, USA. 92:8358-8362, 1995.

54. **Weiner LM**, Clark JI, Ring DB, Alpaugh RK. Clinical Development of 2B1, A Bispecific murine monoclonal antibody targeting c-erbB-2 and FcγRIII. J Hematotherapy. 4:453-456, 1995.

55. **Weiner LM**, Clark JI, Davey M, Li WS, Garcia de Palazzo I, Ring DB, Alpaugh RK. Phase I trial of 2B1, A bispecific monoclonal antibody targeting c-erbB-2 and FcγRIII. Cancer Res. 55:4586-4593, 1995.

56. Clark JI, **Weiner LM**: Biologic treatment of human cancer, Current Problems in Cancer. 19(4):189-262, 1995.

57. Adams GP, **Weiner LM**: Intracellular Single-chain Fv antibodies - A knockout punch for neoplastic cells. Gyn Oncol. 59:6-7, 1995.

58. Haas NB, Schilder RJ, Nash S, **Weiner LM**, Catalano RC, Ozols RF, O'Dwyer PJ: A Phase II trial of weekly infusional 5-fluorouracil in combination with low-dose leucovorin in patients with advanced colorectal cancer. Invest New Drugs. 13:229-233, 1995.

59. Munn DH, Bree AG, Beall AC, Kaviani MD, Sabio H, Schaub RG, Alpaugh RK, **Weiner LM**, Goldman SJ: Recombinant human macrophage colony-stimulating factor in non-human primates: Selective expansion of a CD16+ monocyte subset with phenotypic similarity to natural killer cells. Blood. 88:1215-1224, 1996.

60. Amoroso AR, Clark JI, Litwin S, Hsieh-Ma S, Shi T, Alpaugh RK, Adams GP, Wolf EJ, Ring DB, **Weiner LM**. Binding characteristics and antitumor properties of 1A10 bispecific antibody recognizing gp40 and human transferrin receptor. Cancer Res. 56:113-120, 1996.

61. **Weiner LM**, Alpaugh RK, Amoroso AR, Adams GP, Ring DB, Barth MW. Human neutrophil interactions of a bispecific monoclonal antibody targeting tumor and human FcγRIII. Cancer Immun Immunother. 42:141-150, 1996.

62. Schier R, Bye J,Apell G, McCall A, Adams GP, Malmquist M, **Weiner LM**, Marks JD. Isolation of high-affinity monomeric human anti-c-erbB-2 single chain Fv using affinity driven selection. J Mol Biol. 255:28-43, 1996.

63. Huston JS, George AJT, Adams GP, Stafford WF, Jamar F, Tai M-S, McCartney JE, Opperman H, Heelan BT, Peters AM, Houston LL, Bookman MA, Wolf EJ, **Weiner LM**: Single-chain Fv radioimmunotargeting. J Nuc Med. 40:320-333, 1996.

64. Schier R, McCall A, Adams GP, Marshall KW, Merritt H, Yim M, Crawford RS, **Weiner LM**, Marks JD. Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the complementarity determining regions in the center of the antibody binding site. J Mol Biol. 263:551-567, 1996.

65. von Mehren M, **Weiner LM**: Monoclonal antibody-based therapy, In Current Opinion in Oncology, eds. Abeloff MD, Klastersky J, Rapid Science Ltd, Philadelphia, pp. 8:493-498, 1996.

66. Clark JI, Alpaugh RK, von Mehren M, Schultz J, Gralow JR, Cheever MA, Ring DB, **Weiner LM**. Induction of multiple anti-c-erbB2 specificities accompanies a classical idiotypic cascade following 2B1 bispecific monoclonal antibody treatment. Cancer Immunol Immunother. 44:265-272, 1997.

67. Giantonio BJ, Alpaugh RK, Schultz J, McAleer C, Newton DW, Guedez Y, Kotb M, Vitek L, Persson R, Kelland T, Dohlsten M, Persson B, **Weiner LM**: Superantigen-based Immunotherapy: A Phase I Trial of LS4565, a Monoclonal Antibody-Staphylococcal Enterotoxin A Recombinant Fusion Protein, in Advanced Pancreatic and Colorectal Cancer. J Clin Oncol. 15:1994-2007, 1997.

68. von Mehren M and **Weiner LM**: Interferon-Gamma in Malignant Disease, In The Clinical Application of the Interferons, (1997) eds. Harris and Penny, Chapman & Hall Medical, London pp.315-331.

69. **Weiner LM**, Colarusso P, Goldberg M, Dresler C, Coia LR. Combined modality therapy for esophageal cancer: Phase I trial of escalating doses of paclitaxel in combination with cisplatin, 5-fluorouracil, and high-dose radiation before esophagectomy. Seminars in Oncology. 24 (Suppl 19):S19-93-S19-95, 1997.

70. **Weiner LM**. Biological therapies for gastrointestinal cancers, In Current Opinion in Oncology, (1997) ed. Haller D, Rapid Science Ltd, Philadelphia, 9:373-379.

71. **Weiner LM**, Alpaugh RK, von Mehren M. Redirected cellular cytotoxicity employing bispecific antibodies and other multifunctional binding proteins. Cancer Immunol Immunother. 45:190-192, 1997.

72. Lattanzi JP, Coia LR, Scher RM, Engstrom PF, Hoffman JP, Eisenberg BF, Sigurdson ER, Epstein BE, **Weiner LM**. A prospective Phase I/II study of intra-arterial chemotherapy and escalating doses of radiation therapy for surgically unresectable carcinoma confined to the liver. GI Cancer. 2:111-117, 1997.

73. Alpaugh RK, **Weiner LM**, Persson B, Persson R, Dohlsten M, Kalland T. Superantigen-Based immunotherapy. Drugs Fut. 22(11):126-1230, 1997.

74. **Weiner LM**. Modulating binding affinity to improve the tumor-targeting properties of monomeric single-chain Fv molecules, In Antibody Engineering II. New technology, application and Commercialization. W. Hori (ed). Intl Business Communicating Inc, Southborough MA Vol 1:pp 51-58, 1997.

75. Adams GP, Schier R, McCall AM, Crawford RS, Wolf EJ, **Weiner LM**, Marks JD. Prolonged in vivo tumor retention of a human diabody targeting the extracellular domain of human HER2/neu. Brit J Cancer. 77:1405-1412, 1998.

76. Adams GP, Schier R, Marshall K, Wolf EJ, McCall AM, Marks JD, **Weiner LM**. Increased affinity leads to improved selective tumor delivery of single chain Fv antibodies. Cancer Res. 58:485-490, 1998.

77. Alpaugh RK, Schultz J, McAleer C, Giantonio BJ, Persson R, Burnite M, Nielsen SE, Vitek L, Persson B, **Weiner LM**. Superantigen-targeted therapy: Phase I escalating repeat dose trial of the fusion protein PNU-214565 in patients with advanced gastrointestinal malignancies. Clinical Cancer Res. 4:1903-1914, 1998.

78. Alpaugh RK, **Weiner LM**, Persson R, Persson B. Overview of clinical trials employing antibody-targeted superantigens. Advanced Drug Delivery Reviews. 31(1,2):143-152, 1998.

79. Keller SM, Ryan LM, Coia LR, Dang P, Vaughn DJ, Diggs C, Weiner LM, Benson AB. High dose chemoradiation followed by esophagectomy for adenocarcinoma of the esophagus and gastroesophageal junction: Results of a phase II study of the Eastern Cooperative Oncology Group. Cancer. 83(9):1908-1916, 1998.

80. Alpaugh RK, von Mehren M, Palazzo I, Atkins MB, Sparano J, Schuchter L, Dutcher JP, **Weiner LM**. Phase IB trial for malignant melanoma using R24 monoclonal antibody, interleukin-2/α-interferon. Medical Oncology. 15:191-198, 1998.

81. McCall AM, Amoroso AR, Sautes C, Marks JD, **Weiner LM**. Characterization of anti-mouse FcγRII single-chain Fv fragments derived from human phage display libraries. Immunotechnology. 4:71-87, 1998.

82. Movsas B, Hanlon AL, Lanciano R, Scher RM, **Weiner LM**, Sigurdson ER, Hoffman JP, Eisenberg BL, Cooper HS, Provins S, Coia LR. Phase I dose escalating trial of hyperfractinated

pre-operative chemoradiation for locally advanced rectal cancer. Int J Radiation Oncology Biol Phys. 42:43-50, 1998.

83. McCall AM, Adams GP, **Weiner LM**. Antibody-targeted gene therapy In Gene Therapy of Cancer (1998) Eds: E. Lattime and S. Gerson, Academic Press, San Diego, CA: pp 7:113-124.

84. Amoroso AR, Alpaugh RK, Barth MW, McCall AM, **Weiner LM**. Production and characterization of mice transgenic for the A and B isoforms of human FcγRIII. Cancer Immunol Immunother. 48:443-455, 1999.

85. **Weiner LM**. An overview of monoclonal antibody therapy of cancer. Seminars in Oncology. 26(4)S12:41-50, 1999.

86. **Weiner LM**. Paclitaxel in the treatment of esophageal cancer. Seminars in Oncology. 26/1:106-108, 1999.

87. McCall AM, Adams GP, Amoroso AR, Nielsen UB, Zhang L, Horak E, Simmons H, Schier R, Marks JD, **Weiner LM**. Isolation and characterization of an anti-CD16 single-chain Fv fragment and construction of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers CD16-dependent tumor cytolysis. Molecular Immunology. 36:433-446, 1999.

88. Hudes GR, Lipsitz S, Grem J, Morrisey M, **Weiner L**, Kugler J, Benson A. Phase II study of 5-Fluorouracil, Leucovorin, and interferon-α in the treatment of patients with metastatic or recurrent gastric carcinoma: An Eastern Cooperative Oncology Group Study (E5292). Cancer. 85:290-294,1999.

89. Segal DM, Weiner GJ, **Weiner LM**. Bispecific Antibodies in cancer therapy. Current Opinion in Immunology. 11:558-562, 1999.

90. **Weiner LM**. Monoclonal antibody therapy of cancer. Semin Oncol. 26(5)S14:43-51, 1999.

91. Knox SJ, Goris ML, Tempero M, Weiden PL, Gentner L, Breitz H, Adams GP, Axworthy D, Gaffigan S, Bryan K, Risher DR, Colcher D, Horak ID, **Weiner LM**. Phase II trial of Yttrium-90-Dota-Biotin pretargeted by NR-LU-10 antibody/streptavidin in patients with metastatic colon cancer. Clinical Cancer Research. 6:406-414, 2000.

92. VonMehren M, **Weiner LM**. Monoclonal Antibodies: Clinical Applications-Breast Cancer and other Adenocarcinomas. In Principles and Practice of the Biologic Therapy of Cancer, 3rd Edition, (2000) SA Rosenberg (ed). Lippincott Williams & Wilkins, Philadelphia, PA pp 452-475.

93. **Weiner LM**, Adams GP, von Mehren M. Therapeutic Monoclonal Antibodies: General Principles. In Cancer: Principles and Practice of Oncology, (2000) V. DeVita, S. Hellman, SA Rosenberg (eds). Lippincott Williams & Wilkins, Philadelphia, PA. pp 495-508.

94. **Weiner LM**. Chapter 4: Oncologic Diseases. In Medicine, (2001) A. Myers (ed). Lippincott Williams & Wilkins, Philadelphia, PA. Pp 155-229.

95. von Mehren M, Arlen P, Tsang KY, Rogatko A, Meropol N, Cooper HS, Davey M, McLaughlin S, Schlom J, **Weiner LM**. Pilot study of a dual gene recombinant Avipox vaccine containing both CEA and B7.1 transgenes in patients with recurrent CEA expressing adenocarcinomas. Clin Cancer Res. 6:2219-2228, 2000.

96. Hudes G, Szarka C, Adams A, Ranganathan S, McCauley R, **Weiner L**, Langer C, Litwin S, Yeslow G, Halberr T, Qian M, Gallo JM. Phase I pharmacokinetic trial of perillyl alcohol (NSC 641066) in patients with refractory solid malignancies. Clinical Can Res. 6:3071-3080, 2000.

97. Adams GP, Shaller CC, Chappell LL, Wu C, Horak EM, Simmons HH, Litwin S, Marks J, **Weiner LM**, Brechbiel MW. Delivery of the Alpha-emitting radioisotope Bismuth-213 to solid tumors via single-chain Fv and diabody molecules. Nucl Med Biol. 27:339-346, 2000.

98. Nielsen UB, Adams GP, **Weiner LM**, Marks JD. Targeting of bivalent anti-ErbB2 diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. Cancer Res. 60:6434-6440, 2000.

99. **Weiner, LM**. Bispecific antibodies in cancer therapy. The Cancer Journal. 6(S3):S265-271, 2000.

100. Cheng JD, Rieger PT, von Mehren M, Adams GP, **Weiner LM**. Recent advances in immunotherapy and monoclonal antibody treatment of cancer. Sem Oncol Nursing. 16(4):2-12, 2000.

101. Baselga J, O'Dwyer P, Thor AD, Vokes E, **Weiner LM**. Epidermal growth factor receptor: Potential target for antitumor agents. Monograph, The Center for Biomedical Continuing Education, 2000.

102. **Weiner LM**. The Cancer Protocol Guide. (2000) LM Weiner (ed.) Lippincott Wilkins & Wilkins Healthcare, Philadelphia, PA.

103. **Weiner LM**, Adams GP. New approaches to antibody therapy. Oncogene. 19:6144-6151, 2000.

104. Schilder RJ, Gallo JM, Kindsfather SK, Millenson MM, Bookman MA, Adams AL, Ciccotto S, Boente M, Rosenblum N, Hogan WM, Rogers B, Padavic-Shallers K, **Weiner LM**, Ozols RF. A Phase I trial of multiple cycles of high dose Carboplatin-Paclitaxel-Topotecan with peripheral blood stem cell support as front-line therapy. J Clin Oncol. 19:1183-1194, 2001.

105. Pandya KJ, Yeap BY, **Weiner LM**, Krook JE, Erban JK, Schinella RA, Davis TE. Megestrol and tamoxifen in patients with advanced endometrial cancer: an Eastern Cooperative Oncology Group Study (E4882). Am J Clin Oncol. 24:43-46, 2001.

106. Adams GP, Schier R, McCall AM, Simmons HH, Horak EM, Alpaugh RK, Marks JD, **Weiner LM**. High affinity restricts the localization and tumor penetration of single-chain Fv antibody molecules. Cancer Res. 61:4750-4755, 2001.

107. von Mehren M, Arlen P, Gulley J, Rogatko A, Cooper HS, Meropol NJ, Alpaugh RK, Davey M, McLaughlin S, Beard MT, Tsang KY, Schlom J, **Weiner LM**. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. Clin Cancer Res. 7:1181-1191, 2001.

108. von Mehren M, **Weiner LM**. Adjuvant immunotherapy of breast cancer. In Strategies in Adjuvant Therapy, (2000) J. Kirkwood (ed), Dunitz.

109. O'Dwyer PJ, Manola J, Valone FH, Ryan LM, Hines JD, Wadler S, Haller DG, Arbuck SG, **Weiner LM**, Mayer RJ, Benson AB. Fluorouracil modulation in colorectal cancer: Lack of

improvement with PALA or oral leucovorin or interferon, but enhanced therapeutic index with weekly 24-hour infusional schedule. An ECOG/CALGB Study. J Clin Oncol. 19:2413-2421, 2001.

110. Segal DM, Weiner GJ, **Weiner LM**. Introduction: bispecific antibodies. J Immunological Methods (Special Issue: Bispecific Antibodies). 248:1-6, 2001.

111. Powers, DB, Amersdorfer P, Alix-Poul M, Refaat-Shalaby M, Adams GP, **Weiner LM**, Marks JM. Expression of single chain Fv-Fc fusions in *Pichia Pastoris*. J Immunol Methods. 251: 123-135, 2001.

112. Adams GP, **Weiner LM**. Radioimmunotherapy of solid tumors: From fairytale to reality. Cancer Biotherapy & Radiopharmaceuticals. 16:9-11, 2001.

113. McCall AM, Shahied L, Amoroso AR, Horak EM, Simmons HH, Nielson U, Adams GP, Schier R, Marks JD, **Weiner LM**. Increasing the affinity for tumor antigen enhances bispecific antibody cytotoxicity. J Immunol. 166:6112-6117, 2001.

114. Lewis NL, **Weiner, LM**. Translational Research (Overview of Phase I, II and III Clinical Trials). In The Cancer Handbook. (2001) MR Alison (ed.), Nature Publishing Group, Macmillan Publishers Ltd., England.

115. Lampert C, McCall AM, **Weiner LM**. Antibody-targeted gene therapy. In Gene Therapy of Cancer: Translational Approaches from Preclinical Studies to Clinical Implementation. (2002) EC Lattime (ed.), Academic Press, pp. 81-94.

116. Crul M, De Klerk GJ, Swart M, **Weiner L**, Palmer PA, Bol CJ, Beijnen JH, Schellens JHM. Evaluation of the bioequivalence of tablets and capsules containing the novel anticancer agent R115777 (Zarnestra) in patients with advanced solid tumors. Eur J Drug Metab Pharmacokinet. 27:61-65, 2002.

117. Cheng JD, Dunbrack RL Jr, Valianou M, Rogatko A, Alpaugh RK, **Weiner LM**. Promotion of tumor growth by murine fibroblast activation protein (FAP), a serine protease, in an animal model. Cancer Res. 62:4767-4772, 2002.

118. Lewis NL, Scher R, Gallo JM, Engstrom PF, Szarka CE, Litwin S, Adams AL, Kilpatrick D, Brady D, **Weiner LM**, Meropol NJ. Phase I and pharmacokinetic study of irinotecan in combination with raltitrexed. Cancer Chemother Pharmacol.50:257-265, 2002.

119. Figlin R, Belldegrun A, Crawford J, Lohner M, Roskos L, Yang XD, Foon KA, Schwab G, **Weiner L**. Phase I clinical trial of ABX-EGF, a fully human anti-epidermal growth factor receptor (EGFR) monoclonal antibody (mAb) for patients with advanced cancer. Int J Cancer 87:87, 2002.

120. **Weiner LM**. Beyond neutrophil recovery: manipulation of the tumor microenvironment by GM-CSF to control Cancer. Oncology (Huntingt). 16:7-9, 2002.

121. Lewis NL, **Weiner LM**. Principles of biological therapy. In Clinical Practice of Hematology and Oncology: Presentation, Diagnosis and Treatment. (2003) B Furie, PA Cassileth, MB Atkins, RJ Mayer (eds.), Churchill Livingston/Elsevier Inc, Philadelphia, PA, pp. 376-390.

122. Benz C, Clark G, Conzen S, Dorn R, Fuqua S, Gralow J, Greene G, Helmann R, Hellman S, Lippman M, Rosen N, **Weiner L**. Consensus Statement: Expedition Inspiration Fund for Breast Cancer Research Meeting 2002. Breast Cancer Res Treat. 78:127-131, 2003.

123. VonMehren M, Adams GP, **Weiner LM**. Monoclonal Antibody Therapy for Cancer. Annu Rev Med. 54:343-369, 2003.

124. Cohen SJ, Ho L, Ranganathan S, Abbruzzese JL, Alpaugh RK, Beard M, Lewis NL, McLaughlin S, Rogatko A, Ruixo JJP, Thistle AM, Verhaeghe T, Wang H, **Weiner LM**, Wright JJ, Hudes GR, Meropol NJ. Phase II and pharmacodynamic study of the farnesyl transferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. J Clin Oncol. 21:1301-1306, 2003.

125. **Weiner LM**, Carter P. The rollercoaster ride to anti-cancer antibodies. A recent Keystone meeting highlighted to progress in developing antibody-based therapeutics for cancer. Nat Biotechnol. 21(5): 510-511, 2003.

126. Cheng J, **Weiner LM**. Tumors and their Microenvironments: Tilling the Soil - Commentary. Clin Cancer Res. 9:1590-1595, 2003.

127. Goldberg M, Farm J, Lampert C, Colarusso P, Coia L, Frucht H, Goosenberg E, Beard M, **Weiner LM**. Survival following intensive preoperative combined modality therapy with paclitaxel, cisplatin, 5-fluorouracil, and radiation in respectable esophageal carcinoma: A phase I report. J Thorac Cardiovasc Surg. 126:1168-1173, 2003.

128. Cheng JD, Baab JS, Langer C, Aamdal S, Robert F, Engelhardt LR, Fernberg O, Schiller J, Forsberg G, Alpaugh RK, **Weiner LM**, Rogatko A. Individualized patient dosing in Phase I clinical trials: The role of escalation with overdose control in PNU-214936. J Clin Oncol. 22:602-609, 2004.

129. Foon KA, Yang X-D, **Weiner L**, Beldegrun A, Figlin R, Crawford J, Rowinsky EK, Dutcher JP, Vogelzang NJ, Gollub J, Thompson J, Schwartz G, Bukowski RM, Roskos L, Schwab G. Preclinical and clinical evaluations of ABX-EGF, a fully human anti-epidermal growth factor receptor antibody. Int J Radiation Oncology Biol Phys. 58:984-990, 2004.

130. Kaufman HL, Wang W, Manola J, DiPaola RS, Ko Y-J, Sweeney C, Whiteside TL, Schlom J, Wilding G, **Weiner LM**. Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): A trial of the Eastern Cooperative Oncology Group. J Clin Oncol. 22:2122-2132, 2004.

131. Adams GP, Shaller CC, Dadachova E, Simmons HH, Horak EM, Tesfaye A, Klein-Szanto AJP, Marks JD, Brechbiel MW, **Weiner LM**. A single treatment of ^{90}Y-CHX-A" C6.5 diabody inhibits the growth of established human tumor xenografts in immunodeficient mice. Cancer Res. 64:6200-6206, 2004.

132. Cohen SJ, Gallo J, Lewis NL, Alpaugh RK, Gentner L, Rogatko A, Yeslow G, Schol J, Verhaeghe T, Zannikos P, Palmer PA, **Weiner LM**, Meropol NJ. Phase I and pharmacokinetic study of the farnesyltransferase inhibitor R115777 in combination with Irinotecan in patients with advanced cancer. Cancer Chemother Pharmacol. 53:513-518, 2004.

133. Borghaei H, **Weiner LM**. Oncologic Diseases: In NMS Medicine, 5th Edition, (2004) A Myers (ed). Lippincott Williams & Wilkins, Baltimore, MD, pp. 163-226.

134. Shahied LS, Tang Y, Alpaugh RK, Somer R, Greenspon D, **Weiner LM**. Bispecific minibodies targeting HER2/neu and CD16 exhibit improved tumor lysis when placed in a divalent tumor antigen-binding format. J Biol Chem. 279:53907-53914, 2004.

135. Cheng JD, Adams GP, Robinson MK, **Weiner LM**. Monoclonal Antibodies: In Cancer: Principles and Practice of Oncology, 7th Edition, (2005) VT DeVita, S Hellman, SA Rosenberg (eds). Lippincott Williams & Wilkins, Philadelphia, PA, pp. 445-456.

136. Berger AC, Farma J, Scott WJ, Freedman G, **Weiner L**, Cheng JD, Wang H, Goldberg M. Complete response to neoadjuvant chemoradiotherapy in esophageal carcinoma is associated with significantly improved survival. J Clin Oncol. 23:1-8, 2005.

137. Cheng JD, Valianou M, Canutescu A, Jaffe E, Lee H-O, Wang H, Lai JH, Bachovchin WW, **Weiner LM**. Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. Mol Cancer Ther. 4(3):351-360, 2005.

138. **Weiner LM**, Carter P. Tunable antibodies: A recent Keystone meeting highlighted the progress in generating effective anti-cancer antibodies by manipulating antibody binding affinity and their ability to support ADCC. Nat Biotechnol. 23(5):556-557, 2005.

139. Smirnov DA, Zweitzig, DR, Foulk BW, Miller MC, Doyle GV, Pienta KJ, Meropol NJ, **Weiner LM**, Cohen SJ, Moreno JG, Connelly MC, Terstappen WMM, O'Hara SM. Global gene expression profiling of circulating tumor cells. Cancer Res. 65:4993-4997, 2005.

140. Adams GP, **Weiner LM**. Monoclonal antibody therapy of cancer. Antibody Engineering Focus Issue. Nat Biotechnol. 23:1147-1157, 2005.

141. Horak E, Heitner T, Robinson MK, Simmons HH, Garrison J, Russeva M, Furmanova P, **Weiner LM**, Adams GP, Marks JD. Isolation of scFvs to in vitro produced extracellular domains of EGFR family members. Cancer Biother Radiopharm. 20:603-613, 2005.

142. Snyder L, Astsaturov I, **Weiner LM**. Overview of monoclonal antibodies and small molecules targeting the epidermal growth factor receptor pathway in colorectal cancer. Clin Colorectal Cancer. 5(S2): S71-S80, 2005.

143. Berger AC, Scott WJ, Freedman G, Konski A, **Weiner L**, Cheng JD, Goldberg M. Morbidity and mortality are not increased after induction chemoradiotherapy followed by esophagectomy in patients with esophageal cancer. Semin Oncol. 32(suppl 9): S16-S20, 2005.

144. Henry LR, Goldberg M, Scott W, Konski A, Meropol NJ, Freedman G, **Weiner L**, Watts P, Beard M, McLaughlin S, Cheng JD. Induction Cisplatin and Paclitaxel followed by combination chemoradiotherapy with 5-Fluorouracil, Cisplatin, and Paclitaxel before resection in localized esophageal cancer: A Phase II report. Ann Surg Onc. 13(2):214-220, 2006.

145. **Weiner LM**. Fully human therapeutic monoclonal antibodies. J Immunother. 29:1-9, 2006.

146. Adams GP, Tai M-S, McCartney JE, Marks JD, Houston LL, Stafford III WF, Huston JS, **Weiner LM**. Avidity-mediated enhancement of in vivo tumor targeting by single-chain Fv dimers. Clin Cancer Res. 12(5): 1599-1605, 2006.

147. Scallon BJ, Snyder LA, Anderson GM, Chen Q, Yan L, **Weiner LM**, Nakada MT. A review of antibody therapeutics and antibody-related technologies for oncology. J Immunother 29:351-364, 2006.

148. Binyamin L, Borghaei H, **Weiner LM**. Cancer therapy with engineered monoclonal antibodies. Update on Cancer Therapeutics 1: 147-157, 2006.

149. **Weiner, LM.** Monoclonal antibody therapy for metastatic colorectal cancer. Am J Hem Onc 5(S8): 12-14, 2006.

150. **Weiner LM**, Borghaei H. Targeted therapies in solid tumors: Monoclonal antibodies and small molecules. Human Antibodies 15(3): 103-111, 2006.

151. Movsas B, Diratzouian H, Hanlon A, Cooper H, Freedman G, Konski A, Sigurdson E, Hoffman J, Meropol NJ, **Weiner L**, Coia L, Lanciano R, Stein J, Kister D, Eisenberg B. Phase II trial of preoperative chemoradiation with a hyperfractionated radiation boost in locally advanced rectal cancer. Am J Clin Oncol 29(5): 435-441, 2006.

152. Cohen SJ, Alpaugh RK, Gross S, O'Hara SM, Smirnov DA, Terstappen Louis M. Weiner, M.D., Allard WJ, Bilbee M, Cheng JD, Hoffman JP, Lewis NL, Pellegrino A, Rogatko A, Sigurdson E, Wang H, Watson JC, **Weiner LM**, Meropol NJ. Isolation and characterization of circulating tumor cells in patients with metastatic colorectal cancer. Clin Colorectal Cancer 6:125-132, 2006.

153. Cianfrocca ME, Kimmel KA, Gallo J, Cardoso TM, Brown MM, Hudes G, Lewis NL, **Weiner LM**, Lam GN, Brown SC, Shaw DE, Mazar AP, Cohen RB. Phase I trial of the antiangiogenic peptide ATN-161 (AC-PHSCN-NH_2), A beta integrin antagonist, in patients with solid tumors. Brit J Cancer 94:1621-1626, 2006.

154. Borghaei H, Alpaugh RK, Bernardo P, Palazzo IE, Dutcher JP, Venkatraj U, Wood WC, Goldstein L, **Weiner LM**. Induction of adaptive anti-HER2/neu immune responses in a Phase IB/2 trial of 2B1 bispecific murine monoclonal antibody in metastatic breast cancer (E3194): A trial coordinated by the Eastern Cooperative Oncology Group. J Immunother 30(4): 455-467, 2007.

155. Bechman RA, **Weiner LM**, Davis HM. Antibody constructs in cancer therapy: Protein engineering strategies to improve exposure in solid tumors. Cancer 109(2):170-179, 2007.

156. Atkins MB, Carbone D, Coukos G, Dhodapkar M, Ernstoff MS, Finke J, Gajewski TF, Gollob J, Jones P, Lotze M, Storkus W, **Weiner LM**. Report on the iSBTc mini-symposium on biologic effects of targeted therapeutics. J Immunother 30(6): 577-590, 2007.

157. **Weiner LM**. Building better magic bullets: improving unconjugated monoclonal antibody therapy for cancer. Nature Reviews Cancer 7:701-706, 2007.

IN PRESS:

1. Carson III WE, Allen A, **Weiner LM**, Cheever MA, Fox BA, Keilholz U, Wigginton JM, Sondel PM, Atkins MB, Hwu P. Immune therapy comes of age: Overview of the 21st annual meeting and associated programs of the International Society for the Biological Therapy of Cancer (iSBTc). (In press): Expert Opinion in Biological Therapy 2006.

2. **Weiner LM**, Dhodapkar MV, Ferrone S. Monoclonal antibodies for cancer immunotherapy. (In press): Lancet 2007.

3. **Weiner LM.** Immune Prevention of Cancer – Future, not Fantasy. *Issues and Insights* (In Press): Cancer Prevention newsletter, 2007.

4. Cohen SJ, Engstrom PF, Lewis NL, Langer CJ, McLaughlin S, Beard M, **Weiner LM**, Meropol NJ. Phase I study of oxaliplatin and capecitabine in combination with the proteasome inhibitor bortezomib in patients with advanced solid tumors. (In Press): Am J Clin Oncol, 2007.

5. Konski AA, Cheng JD, Goldberg M, Li T, Maurer A, Yu JQ, Haluszka O, Scott W, Meropol NJ, Cohen SJ, Freedman G, **Weiner LM**. Correlation of molecular response as measured by 18-FDG PET with outcome after chemoradiation in patients with esophageal carcinoma. (In Press): Int J Rad Onc Biol Phys 2007.

6. **Weiner LM**, Belldegrun AS, Crawford J, Tolcher AW, Lockbaum P, Arends RH, Navale L, Amado RG, Schwab G, Figlin RA. Dose and schedule study of panitumumab monotherapy in patients with advanced solid malignancies. (In Press): Clin Cancer Res 2007.

7. Robinson MK, Borghaei H, Adams GP, **Weiner LM**. Pharmacology of Biotherapeutics: Monoclonal Antibodies: In Cancer: Principles and Practice of Oncology, 8th Edition, (2007) VT DeVita, S Hellman, SA Rosenberg (eds). Lippincott Williams & Wilkins, Philadelphia, PA. (In Press).

8. **Weiner LM**, Meropol NJ, Cohen SJ, Narra K, Lee H-O, Mullins SR, Strzempkowski-Brun B, Magalong K, Egleston BL, Christiansen V, McKee P, Cheng J. Phase II trial of single agent Val-boroPro (Talabostat) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. (In Press): Cancer Biol Ther 2007.

SUBMITTED:

1. Tang Y, Lou J, Alpaugh RK, Marks JD, **Weiner LM**. Regulation of antibody-dependent cellular cytotoxicity by IgG intrinsic and apparent affinity for target antigen. Submitted.

2. Kirkwood JM, Lee S, Moschos S, Albertini MR, Michalak JC, Sander C, Whiteside T, **Weiner LM**. Immunogenicity and anti-tumor effects of vaccination with a multi-epitope lineage antigen peptide vaccine with or without GM-CSF and/or interferon alfa-2b in patients with advanced metastatic melanoma: the ECOG Phase II trial E1696. Submitted.

3. Cohen SJ, Alpaugh RK, Palazzo I, Meropol NJ, Rogatko A, Xu Z, Hoffman JP, **Weiner LM**, Cheng JD. Fibroblast activation protein and its relationship to clinical outcome in pancreatic adenocarcinoma. Submitted.

ABSTRACTS:

1. **Weiner LM**, Huang B-S, Smith KM, Schwartz RS, and Stollar BD. Antibody Recognition of the Helical Conformation of Double-Stranded DNA and DNA Analogues. Presented to the Fifth International Congress of Immunology, Kyoto, Japan; August, 1983.

2. **Weiner LM** and Comis RL. Phase I Trial of Murine Monoclonal Antibody Administration Preceded by Recombinant Gamma-Interferon Therapy in Patients with Advanced Gastrointestinal Carcinoma. Proceedings, ASCO, Los Angeles, CA; May, 1986.

3. **Weiner LM**, Steplewski Z, Koprowski H, and Comis RL. Effects of Gamma- Interferon Therapy on Monocyte Cytotoxicity. Presented to Congress on Research in Lymphokines and Other Cytokines, Boston, MA; August, 1986.

4. Schwob VS, **Weiner LM**, Hudes G, Ratech H. Extranodal Non-T Cell Lymphoblastic Lymphoma in Adults: A New Clinicopathologic Entity. Presented to International Academy of Pathology, 1986.

5. **Weiner LM**, Steplewski Z, Koprowski H, Comis RL. Superiority of Low over High Dose Gamma-Interferon Therapy in A Phase I Clinical Trial of rIFN-γ Plus Murine Monoclonal Antibody Therapy. Presented to ASCO, Atlanta, GA; May, 1987.

6. **Weiner LM**, Ring D. Enhancement of in vitro Human LAK Cell Activity by Tumor Binding Monoclonal Antibodies. Presented to AACR, Atlanta, GA; May, 1987.

7. Daugherty JP, **Weiner LM**. Augmentation of IL-2-Induced IL-2 Receptor Expression by IFN-γ. Presented to AACR, Atlanta, GA; May, 1987.

8. **Weiner LM**, Moldofsky P, Gatenby R, and Comis RL. Combination Therapy with Murine Monoclonal Antibody 17-1A and Immunomodulatory Doses of Interferon-γ in Advanced Colorectal Carcinoma. Presented to AFCR, Eastern Section, October, 1987.

9. Cornfeld M, Comis RL, Hudes G, **Weiner LM**, Catalano RB, O'Dwyer P. Phase I Study of 5-Fluorouracil (5-FU) with Leucovorin (LV) and Cisplatin (DDP) in Patients (Pts) with Advanced Cancer. Presented to ASCO, May 1988.

10. **Weiner LM**, Moldofsky PJ, Gatenby RA, O'Dwyer J, Comis RL. Phase II Trial of Interferon-γ and Monoclonal Antibody 17-1A in Colorectal Carcinoma. Proceedings, May ASCO, 1988.

11. **Weiner LM**, O'Dwyer J, Zintak HS, Bauer EJ, Konrad M, Groves ES, Comis RL. Toxicities of Low Doses of Recombinant Ricin A Chain Immunotoxin in a Phase I Clinical Trial. Proceedings, ASCO, May 1988.

12. **Weiner LM**, O'Brien J, Kitson J, Comis RL. Divergent In Vitro and In Vivo of Recombinant Interferon-γ (rIFN-γ) on Antibody-Dependent Cellular Cytotoxicity (ADCC). Presented to AACR, May 1988.

13. **Weiner LM**, O'Dwyer J, Zintak HS, Bauer EJ, Konrad M, Groves ES, Comis RL. Toxicities of Low Doses of Recombinant Ricin A Chain Immunotoxin in a Phase I Clinical Trial. Presented to International Symposium on Immunotoxins, June 1988.

14. Heda GD, **Weiner LM**, Farber A, Yeh J, Schmaier AH. Gamma-Interferon Increased C1 Inhibitor Levels In Vitro and In Vivo. Presented to American Federation for Clinical Research, April 1989.

15. **Weiner L**, Krigel R, Padavic K, Kitson J, Watts P, Litwin S. Potentiation of Interleukin-2 Effects by γ-Interferon in a Phase I Clinical Trial. Presented to ASCO, May 1989.

16. **Weiner L**, Hudes G, Kitson J, Walczak J, O'Dwyer PJ. Effects of 5-Fluorouracil (5-FU) and PALA Chemotherapy on In Vitro Effector Cell Cytotoxicity. Proceedings, ASCO, May 1989.

17. Garcia I, **Weiner L**. Cytotoxic Effects of Bispecific Anti-(tumor/Fcγ receptor) Monoclonal Antibodies. Presented, AACR, May 1989.

18. **Weiner L**, Kitson J, Groves E. The Toxicity of Ricin A Chain Immunoconjugates May Be Due to Binding to Immunoglobulin Fc (Fcγ) Receptors. Presented, AACR, May 1989.

19. **Weiner LM**, Garcia-Palazzo I, Kitson J, Gercel-Taylor C. Promotion of In Vitro Tumor Lysis by Bispecific Monoclonal Antibodies that Bind Tumor and the Fc_ Receptor (FcγR III) Expressed by Human Large Granular Lymphocytes. Presented, Targeted Cellular Cytotoxicity, Technical Resources, Inc., October 1989.

20. **Weiner LM**, Garcia-Palazzo I, Kitson J, Gercel-Taylor C. Promotion of In Vitro Tumor Lysis by Bispecific Monoclonal Antibodies that Bind Tumor and the Fcγ Receptor (FcγR III) Expressed by Human Large Granular Lymphocytes. Presented, Society for Biologic Therapy, November 1989.

21. **Weiner LM**, Garcia-Palazzo I, Kitson J, Stanfield J, Waas J, Gercel-Taylor C. Destruction of Multicellular Human Tumor Spheroids by Lymphokine-Activated Killer (LAK) Cells Requires Bispecific Monoclonal Antibody Targeting. Presented, AACR, May 1990.

22. **Weiner LM**, Kitson J, Padavic K, Litwin S, Perry P, Krigel RL. Dose-Dependent Effects of γ-Interferon Treatment on Interleukin-2 Therapy-Induced LAK Activity. Presented, AACR, May 1990.

23. Garcia-Palazzo I, Kitson J, Gercel-Taylor C, **Weiner LM**. Human Lymphocyte Trafficking in Immunodeficient Mice. Presented, AACR, May 1990.

24. **Weiner LM**, Haller DG, Kirkwood J, Willson JV, Walsh C, Khazaeli MB, Harvey E. Phase II Trial of Repetitive Murine Monoclonal Antibody Therapy in Pancreatic Carcinoma. An ECOG Trial. Presented, ASCO, May 1991.

25. **Weiner LM**, Holmes M, Garcia dePalazzo IE. Bispecific Antibody Promotion of Tumor Lysis is Mediated in Whole Blood. Presented, AACR, May 1991.

26. Garcia dePalazzo IE, Gercel-Taylor C, Kitson J, Adams S, **Weiner LM**. Neural Cell Adhesion Molecule (N-CAM) Interactions Promote Lysis of Tumor by Bispecific Antibody Redirected Lymphocytes. Presented, AACR, May 1991.

27. Krigel R, Padavic K, **Weiner L**, Comis R. Phase I Study of Sequential Recombinant Tumor Necrosis Factor (rTNF) and Recombinant Interleukin-1 (rIL-2). Proc Annu Meet Am Assoc Cancer Res, 32:A1597, 1991.

28. O'Dwyer PJ, Schilder RJ, Nash S, Haas NB, **Weiner LM**, Catalano RC, Comis RF. Phase II Trial of Weekly Infusional 5-Fluorouracil and Leucovorin in Colorectal Cancer. Proc Annu Meet Am Assoc Cancer Res 32:A1101, 1991.

29. **Weiner LM**, Sundareshan P, Wong A, Garcia de Palazzo IE. Mutated Epidermal Growth Factor Receptor (EGFR) as a Novel Target for Immunotherapy of Lung Cancer. Presented, ASCO, 1992.

30. Lee G, **Weiner L**, Ring D, Holmes M, Alpaugh K, Garcia de Palazzo I. Effective Targeting of Human Lymphocytes and Macrophages to Tumor by a Bispecific Monoclonal Antibody (BsMAb) Binding Human c-erbB-2 and FcγRIII. Presented, AACR, 1992.

31. **Weiner LM**, Sundareshan P, Wong A, Garcia de Palazzo IE. Mutated Epidermal Growth Factor Receptor (EGFR) as a Novel Target for Immunotherapy of Lung Cancer. Presented, AACR, 1992.

32. Bookman MA, Schultz J, Giantonio B, **Weiner LM**, Adams G, Houston L, Ring D, Apell J, Huston JS, Stafford WF. Immunotoxins directed against c-erbB-2: Limited activity due to poor internalization. Third Intl Symposium on Immunotoxins. Orlando, FL, June 19-21, 1992.

33. McCartney JE, Tai M-S, Keck P, Jin D, Stafford III WF, **Weiner LM**, Bookman MA, Oppermann H, Houston LL, Huston JS. Single-chain Fv analogues with C-terminal cysteine (sFv') prepared by direct expression from Escherichia coli: Recovery of bivalent and bispecific 741F8 anti-c-erbB-2 and 26-10 anti-digoxin $(sFv')_2$ dimers. Third IBC Conference on Antibody Engineering. San Diego, CA, December 14-16, 1992.

34. Adams GP, Huston JS, Houston LL, Fand I, Bookman MA, **Weiner LM**. In vivo tumor specificity of monomeric and dimeric forms of ^{125}I-741F8-sFv, a 25 kd single-chain Fv molecule. AACR, presented, 1993.

35. Garcia de Palazzo IE, Richeson A, **Weiner LM**. Binding characteristics of the bispecific monoclonal antibody 2B1. AACR, submitted 1993.

36. Alpaugh K, Li W, Holmes M, **Weiner LM**. Induction of a differentiated macrophage phenotype in a phase I study of recombinant macrophage-colony stimulating factor and recombinant gamma interferon. AACR, presented, 1993.

37. **Weiner LM**, Li W, Catalano RB, Kaye J, Padavic K, Alpaugh RK. Phase I trial of recombinant macrophage-colony stimulating factor (M-CSF) and recombinant gamma-interferon (γ-IFN): peripheral blood mononuclear phagocyte proliferation and differentiation. ASCO, presented, 1993.

38. Alpaugh RK, Li W, Holmes M, **Weiner LM**. Sustained monocytosis with induced differentiated phenotype in a Phase I study of recombinant macrophage-colony stimulating factor and recombinant gamma interferon. AACR, presented, 1993.

39. Adams GP, Huston JS, Houston LL, Fand I, Bookman MA, **Weiner LM**. In vivo tumor specificity of monomeric and dimeric forms of ^{125}-I741F8-sFv, a 25 K_d single-chain Fv molecule. International Conference on Monoclonal Antibody Immunoconjugates for Cancer. San Diego, CA, March 18-20, 1993.

40. Adams GP, Huston JS, Houston JL, Fand I, Bookman MA, **Weiner LM**. In vivo tumor specificity of monomeric and dimeric forms of ^{125}I-741F8-sFv, a 25 K_d single chain Fv molecule. FASEB/AACR Meeting, Denver, CO, May, 1993.

41. **Weiner LM**, McCartney JE, Tai M-S, Oppermann H, Liu S, Stafford III WF, Bookman MA, Fand I, Apell J, Laminet A, Houston LL, Adams GP. Specific in vivo tumor targeting by monovalent and divalent forms of the anti-c-erbB-2 single-chain Fv molecule 741F8. Fourth Annual International Conference on Antibody Engineering, Coronado, CA, 1993.

42. Huston JS, Adams GP, McCartney JE, Tai M-S, Jin D, Hudziak R, Oppermann H, Stafford III WF, Liu S, Fand I, Laminet A, Bookman MA, Houston LL, **Weiner LM**. Refolding and characterisation of single-chain Fv analogues having C-terminal cysteine (sFv'): physicochemical behaviour in vitro and tumor localization in vivo of monovalent sFv' and bivalent (sFv')2 species directed to the c-erbB-2 tumor antigen and digoxin. 10th International Hammersmith Meeting, "Advances in the Applications of Monoclonal Antibodies in Clinical Oncology." Paphos, Cyprus, May 3-5, 1993. Abstracts, p. 11.

43. **Weiner LM**, Ring D, Li W, Palazzo IE, Davey M, Rivera V, Alpaugh RK. Phase I trial of a bispecific murine monoclonal antibody targeting c-erbB-2 and CD16. Presented AACR, 1994.

44. Adams GP, McCartney JE, Wolf EJ, Eisenberg J, Tai M-S, Huston JS, Bookman MA, Stafford WF, Houston LL,, **Weiner LM**. Effects of dose and schedule on tumor targeting and specificity of anti-c-erbB-2 single-chain Fv Molecules.Presented AACR, 1994.

45. **Weiner LM**, Ring D, Li W, Palazzo IE, Davey M, Rivera V, Alpaugh RK. Phase I trial of 2B1, a bispecific murine monoclonal antibody targeting c-erbB-2 and CD16. Presented ASCO, 1994.

46. Adams GP, McCartney J, Wolf EJ, Eisenberg J, Huston JS, Stafford III WF, Bookman MA, Houston LL, **Weiner LM**. Optimization of the in vivo tumor targeting of anti-c-erbB-2 single-chain Fv fragments. International Conference on Monoclonal Antibody Immunoconjugates in Cancer, San Diego, CA, March 3-5, 1994.

47. Huston JS, McCartney JE, tai M-Sk, Adams GP, Hudziak R, Oppermann H, Stafford III WF, Liu S, Fand I, Laminet AA, Bookman MA, Houston LL, **Weiner LM**. Single-chain Fv fusions with C-terminal cysteine peptidfes (sFv'): Divalent anti-c-erbB-2 $(sFv')_2$ shows improved solution behavior in vitro and enhanced tumor localization in vivo compared to monovalent Fab or sFv. Fifth Conference on radioimmunodetection and Radioimmunotherapy of Cancer. Princeton, NJ, October 6-8, 1994.

48. Huston JS, George AJT, Jamar F, Adams GP, Tai M-S, McCartney J, Houston LL, Oppermann H, Peters AM, **Weiner LM**. Perfecting Single-Chain Fv imaging agents. 12th International Hammersmith Meeting, "Advances in the Applications of Monoclonal Antibodies in Clinical Oncology." Molyvos, Lesvos, Greece, May 23-26, 1994.

49. **Weiner LM**, Schier R, Adams GP, Wolf EJ, Bookman MA, Appell G, Houston LL, Huston JS, Stafford W, Marks JD. Tumor targeting properties of anti-c-erbB-2 human single chain Fv molecules prepared from a recombinant phage display library. Presented AACR, 1995.

50. Clark JI, Gralow JR, Alpaugh RK, Disis ML, Cheever MA, Ring D, **Weiner LM**. Bispecific monoclonal antibody-promoted induction of the idiotypic cascade in a Phase I clinical trial. Presented AACR, 1995.

51. Liu S, Tai M-S, McCartney J, Oppermann H, Hudziak RM, Houston LL, **Weiner LM**, Huston JS, Stafford WF. Physical characterization of recombinant anti-c-erbB-2 741F8 single-chain Fv antibody dimer $(sFv')_2$ and analysis of its interaction with recombinant extracellular domain of c-erbB-2 oncogene product by analytical ultracentrifugation. Biophysical Journal 68, A407 (Th-Pos229), 1995. 39th Annual Meeting of the Biophysical Society, San Francisco, CA, February 12-16, 1995.

52. Adams GP, McCartney JE, Wolf EJ, Huston JS, Bookman MA, Stafford WF, Houston LL, **Weiner LM**. Effects of iodination stability on the tumor targeting properties of divalent anti-c-erbB-2 single-chain Fv $(sFv')_2$. Proc Amer Assoc Cancer Res 36:484 (#2884), 1995.

53. George AJT, Jamar F, Tai MS, Heelan BT, Adams GP, McCartney JE, Houston LL, **Weiner LM**, Opperman H, Peters AM, Huston JS. Radiometal labeling of recombinant single-chain Fv antibodies by a genetically engineered, minimal chelation site. Third Annual Congress of the British Society of Immunology, Brighton. UK, Immunology 86(S1):#5.18, December, 1995.

54. Adams GP, McCartney JE, Wolf EJ, Tai M-S, Schier R, Stafford WF, Marks JD, Bookman MA, Huston JS, **Weiner LM**. Influence of avidity on the tumor retention of monospecific and bispecific anti-c-erbB-2 single-chain Fv dimers. Proc Amer Assoc Cancer Res 37:472 (#3217), 1996.

55. McCall AM, Schier R, Amoroso A, Sautes C, Adams GP, Marks JD, **Weiner LM**. Production of bispecific single-chain Fvs $(sFv')_2$ specific for the oncogene product c-erbB-2 and human CD16/mouse FcγRII/III using recombinant phage display libraries. Proc Amer Assoc Cancer Res 37:472 (#3218), 1996.

56. Alpaugh K, Giantonio B, Newton D, Schultz J, Vitek L, Dohlsten M, Kalland T, Movert R, Kotb M, **Weiner L**. Immunological effects of treatment with LS4565, a superantigen-based immunoconjugate. Proc Am Assoc Cancer Res 37:466, 1996.

57. Giantonio BJ, **Weiner LM**, Alpaugh RK, McAleer C, Bonjo C, Schultz JF, Shannon BT, Vitek L, Dohlsten M, Kalland T, Persson B. Superantigen based immunotherapy: A Phase I trial of the staphylococcal enterotoxin A (SEA) immunoconjugate LS4565 in patients (PTS) with advanced pancreatic and colorectal cancer. Proc Amer Soc Clin Oncol 15:1379, 1996.

58. Huston JS, Tai M-S, McCartney JE, Oppermann H, Adams GP, Wolf EJ, **Weiner LW**, Bookman MA, Houston LL, Stafford WF, George AJT, Jamar F, Heelan BT, Peters AM. Single-chain Fv immunotechnology. 1996 Keystone Symposium on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites, Taos, NM, 1996.

59. Adams GP, Schier R, McCartney JE, McCall AM, Wolf EJ, Tai M-S, Stafford WF, Houston LL, Huston JS, Marks JD, **Weiner LM**. Influence of affinity, avidity and size on the tumor retention of anti-c-erbB-2 single-chain Fv molecules. 1996 Keystone Symposium on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites, Taos, NM, 1996.

60. **Weiner LM**, Alpaugh RK, Clark JI, Garcia de Palazzo I, Schultz J, Gralow J, Cheever MA, Hsieh-Ma S, Ring DB. Clinical development of 2B1 bispecific antibody: From the bench to the bedside- and back again. 1996 Keystone Symposium on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites, Taos, NM, 1996.

61. von Mehren M, Wolf EJ, Amoroso EJ, Barth M, Franke TF, Adams GP, Ravetch JS, **Weiner LM**. Human targets for bispecifc antibody-directed therapeutics in FcγRIII transgenic mice. Proc Amer Assoc Cancer Res 37:444 (#3029), 1996.

62. **Weiner LM**, McAleer C, Schultz J, Persson B, Persson R, Dohlsten M, Vitek L, Giantonio BJ, Nielsen SE, Alpaugh RK. Superantigen-targeted therapy: Phase I escalating repeat dose trial of PNU-214565, A fusion protein composed of staphylococcal enterotoxin A (SEA) and C242 antibody Fab fragment in patients with advanced gastrointestinal malignancies. Proc Am Soc Clin Onc 16:436a (#1560), 1997.

63. **Weiner LM**, Colarusso P, Coia LR, Goldberg M, Dresler C, Frucht H, Goosenberg E, Green F, Schmidt K. Combined modality therapy for esophageal cancer: Phase I trial of escalating doses of Taxol in combination with Cisplatin, Fluorouracil and 60 Gy radiation prior to esophagectomy. Proc Am Soc Clin Onc 16:309a (#1101), 1997.

64. Zalcberg JR, Ibrahaim J, Johnston PG, Locker GY, O'Dwyer PJ, **Weiner LM**, Hochster HS, Rao S, Benson AB. ECOG Phase II study of Tomudex in advanced colorectal cancer. Proc Am Soc Clin Onc 16:268a (#951), 1997.

65. Movsas B, Hanlon A, Lanciano R, Scher R, **Weiner L**, Sigurdson E, Hoffman J, Cooper HS, Provins S, Coia L. Phase I dose escalating trial of hyperfractinated pre-operative chemoradiation for locally advanced rectal cancer. IJROBP 39(2):168 (A68), 1997.

66. Calvert PM, Gallo JM, Ciccotto S, Babb J, Rogatko A, Johnson RK, Green F, Hoffman J, O'Dwyer PJ, **Weiner LM**. Phase I clinical and pharmacokinetic study of 5-Fluorouracil, calcium leucovorin and topotecan in patients with advanced cancer, using a novel dose escalation strategy. Proc Am Soc Clin Onc 17:201a (#776), 1998.

67. Langer C, McAleer C, Millenson M, Rosvold E, Bonjo C, Engstrom P, Scher R, Haas N, von Mehren M, **Weiner L**. Phase II evaluation of docetaxel 9D) and carboplatin © in advanced malignancy: Stratification of patient cohorts based on prior treatment (TX) status yields useful results. Proc Am Soc Clin Onc 17:204a (#787), 1998.

68. Hudes G, Schol J, Baab J, Rogatko A, Bol K, Horak I, Langer C, Goldstein L, Szarka C, Meropol N, **Weiner L**. Phase I clinical and pharmacokinetic trial of the farnesyltransferase inhibitor R115777 on a 21-day dosing schedule. Proc Am Soc Clin Onc 18:156a (#601), 1999.

69. Russin M, Alpaugh K, Schultz J, McAleer C, Vaders L, Persson B, Persson R, Kopreski M, **Weiner L**. A phase I trial of C242 Fab-staphylococcal enterotoxin a (SEA) superantigen fusion protein PNU-214565 with dose escalation based upon plasma concentrations of SEA antibodies. Proc Am Soc Clin Onc 18:449a (#1731), 1999.

70. Lampert C, Colarusso P, Goldberg M, Coia L, Dresler C, Frucht H, Goosenberg E, Beard M, **Weiner LM**. Survival following intensive preoperative combined modality therapy with Paclitaxel, Cisplatin, 5-Fluorouracil and radiation in resectable esophageal carcinoma. Proc Am Soc Clin Onc 18:251a (#962), 1999.

71. Cheng JD, Knox SJ, Tempero M, Weiden PL, Horak ID, Gentner L, Bryan K, Gaffigan S, Shaller C, Adams GP, **Weiner LM**. Phase II trial of NR-LU-10 pretargeted delivery of ^{90}yttrium in patients with metastatic colon cancer. Proc Am Soc Clin Onc 18:436a (#1679), 1999.

72. von Mehren M, Davey M, Rivera V, Yeslow G, Gillon T, Alpaugh K, Cheng J, Meropol N, Rosvold E, Scher R, Cooper H, Schlom J, **Weiner LM**. Phase I trial with ALVAC-CEA B7.1 immunization in advanced CEA-expressing adenocarcinomas. Proc Am Soc Clin Onc 18:437a (#1686), 1999.

73. Alpaugh RK, Simmons H, Adams G, **Weiner LM**. Altering the tumor microenvironment through antibody targeting. Proceedings from IBC Antibody Engineering Conference, December 6-9, 1999.

74. Von Mehren M, Davey M, Susan M, Beard M, Meropol NJ, Langer CJ, Cooper H, Schlom J, **Weiner LM**. Phase I study of vaccine therapy with ALVAC-CEA B7.1 and GM-CSF (G) in patients (pts) with advanced CEA-expressing cancers. Proc Am Soc Clin Onc 19:480a (#1883), 2000.

75. Obasaju C, Schol J, von Mehren M, Engstrom P, Millenson M, Haas N, Huang C, Schilder R, **Weiner L**, Langer C. Final results of Phase I trial of Docetaxel (D) and Carboplatin (C) in advanced malignancy: Patient stratification based on prior treatment (Tx) status is crucial. Proc Am Soc Clin Onc 19:211a (#821), 2000.

76. Adams GP, Shaller CS, Horak EM, Simmons HH, Dadachova K, Chappell LL, Wu C, Marks JD, Brechbiel MW, **Weiner LM**. Radioimmunotherapy of established solid tumor xenografts with

alpha and beta emitter-conjugated anti-HER2/*neu* single-chain Fv (scFv) and diabody molecules. Eighth Conf. on Radioimmunodetection and Radioimmunotherapy of Cancer, 2000.

77. **Weiner LM**. Novel Approaches to the Immunotherapy of Colorectal Cancer and Other Solid Tumors. Keystone Symposia Abstract Book: Molecular Medicine of Colorectal Cancer, Taos, NM 2001; pg. 34.

78. Horak EM, Heitner T, Garrison JL, Simmons HH, Alpaugh K, Amoroso AR, Marks JD, **Weiner LM**, Adams GP. Engineering bispecific single-chain Fv molecules to alter signaling of the EGF receptor family. Proc Amer Assoc Cancer Res 42:774 (#4153), 2001.

79. Alpaugh RK,, Simmons HH, Horak EM, Amoroso AR, Adams GP, Marks JD, **Weiner LM**. Altering the cellular milieu at tumor sites using an immunochemotaxin fusion protein of the inflammatory-inducing complement degradation fragment C5a. Proc Amer Assoc Cancer Res 42:290 (#1563), 2001.

80. Cheng JD, Simmons H, Horak E, Dunbrack R, Valinau M, Alpaugh RK, **Weiner LM**. Generation of antibodies targeting murine fibroblast activation protein (FAP), a tumor stromal fibroblast protease. Proc Amer Assoc Cancer Res 42:948 (#5094), 2001.

81. Movsas B, Hanlon AL, Freedman G, Hoffman J, Sigurdson E, Meropol N, **Weiner L**, Coia L, Lanciano R, Cooper H, Black S, Kister D, Eisenberg B. Phase II trial of preoperative chemoradiation with a hyperfractionated RT boost in locally advanced rectal cancer. Intl J Radiat Oncol Biol Phys 54(3):10 (#12), 2001.

82. Figlin R, Belldegron A, Lohner M, Roskos L, Yang X-D, Schwab G, **Weiner L**. ABX-EGF: A fully human anti-EGF receptor antibody in patients with advanced cancer. Proc Am Soc Clin Oncol 20:276a (#1102), 2001.

83. Szarka CE, Babb JS, Cheng JD, Meropol NJ, **Weiner LM**, Engstrom PF, Rogatko A, Lewis NL, Adams A, Gallo JM, Lenaz L, Sun S. Phase I study of Rubitecan and Gemcitabine in patients with unresectable pancreas cancer. Proc Am Soc Clin Oncol 20:135b (#2289), 2001.

84. Figlin R, Belldegrun A, Crawford J, Lohner M, Roskos L, Yang X-D, Schwab G, **Weiner L**. Clinical Results: A fully human anti-Epidermal Growth Factor Receptor (EGFr) antibody in patients with advanced cancer. AACR-NCI-EORTC International Conference. Miami Beach, FL 2001 (#654).

85. **Weiner, LM**. Therapeutic bispecific antibodies. European J Cancer 37(S3):S55(#91), 2001.

86. Shahied LS, Horak EM, Simmons HH, **Weiner LM**. Construction and characterization of bispecific minibody structures to enhance anti-tumor immune response. Proc Am Assoc Cancer Res 43:969 (#4800), 2002.

87. Horak EM, Shahied LS, Shaller CC, Tesfaye A, Simmons HH, Alpaugh RK, Greer NB, Heitner T, Garrison JL, Marks JD, **Weiner LM**, Adams GP. Production and evaluation of bispecific single-chain Fv molecules that target HER2/neu and HER3. Proc Am Assoc Cancer Res 43:971 (#4810), 2002.

88. Cheng JD, Valianou M, Nakajima P, Renner K, Rogatko A, Alpaugh RK, **Weiner LM**. Promotion of tumor growth by murine fibroblast activation protein (FAP), a serine protease. Proc Am Assoc Cancer Res 43:1135 (#5624), 2002.

89. Kaufman HL, Wang W, Manola J, DiPaola RS, Ko Y-J, Williams SD, Whiteside T, Schlom J, Wilding G, **Weiner LM**. Prime/boost vaccination using poxviruses expressing PSA in DO prostate cancer: preliminary results of ECOG 7897, a randomized phase II clinical trial. Proc Am Soc Clin Oncol 21:4a (#12), 2002.

90. Goldberg M, Lampert C, Colarusso P, Coia L, Farma J, Frucht H, Goosenberg E, Beard M, **Weiner LM**. Survival following intensive preoperative combined modality therapy with paclitaxel, cisplatin, 5-fluorouracil and radiation in resectable esophageal carcinoma - A Phase I report. Proc Am Soc Clin Oncol 21:154a (#612), 2002.

91. Cohen SJ, Ho L, Ranganathan S, Lewis N, Rogatko A, **Weiner LM**, Alpaugh RK, Abbruzzese JL, Beard M, McLaughlin S, Wright JJ, Verhaeghe T, Hudes GR, Meropol NJ. Phase II and pharmacodynamic trial of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. Proc Am Soc Clin Oncol 21:137a (#545), 2002.

92. Horak EM, Robinson MK, Heitner T, Garrison JL, Shahied LS, Shaller CC, Tesfaye A, Simmons HH, Alpaugh RK, Greer NB, Marks JD, **Weiner LM**, Adams GP. Targeting the epidermal growth factor receptor family with heterodimeric single-chain FV molecules. Keystone Symposium-Antibody Based Therapeutics for Cancer (B4):43 (#023), 2/4-9, 2003.

93. Shahied LS, Alpaugh RK, Simmons HH, Horak EM, Somer RA, Shaller CC, Greer NB, Lee JS, **Weiner LM**. Investigation of bispecific minibodies with monovalent or divalent tumor antigen binding properties. Keystone Symposium-Antibody Based Therapeutics for Cancer (B4):61 (#218), 2/4-9, 2003.

94. **Weiner LM**, Shahied L, McCall A, Marks J. Selective manipulation of immune response using antibody-based fusion proteins. Keystone Symposium-Antibody Based Therapeutics for Cancer (B4):46 (#032), 2/4-9, 2003.

95. Alpaugh RK, Langer CH, Robert F, Schiller J, Kopreski M, Rogatko A, Forsberg G, **Weiner LM**. Phase II study of amotumoab maferatox (ABR) 214936 in advanced NSCLC: Results of a multi-institutional open label repeat dose, dose trial with pt-specific dose escalation. Proc Am Soc Clin Oncol 22:295 (#2611), 2003.

96. Cohen SJ, Alpaugh RK, Allard J, Cheng JD, Gross ST, Lewis NL, Rogatko A, Sigurdson E, **Weiner LM**, Meropol NJ. Isolation and characterization of circulating tumor cells in patients with colorectal cancer. Proc Am Soc Clin Oncol 22:295 (#1186), 2003.

97. Meropol, NJ, Berlin J, Hecht JR, Croghan G, Patnaik A, **Weiner LM**, Sweed M, Wiens B, Caron D, Visonneau S. Multicenter study of ABX-EGF monotherapy in patients with metastatic colorectal cancer. Proc Am Soc Clin Oncol 22:295 (#1026), 2003.

98. Kirkwood JM, Lee S, Land S, Slattery K, Mascari R, Whiteside T, **Weiner L**. E1696: Phase II trial of multi-epitope peptide vaccination for melanoma with MGT (MART-1 (27-35), gp 100 (209-217, 210M), and tyrosinase (368-376, 370D), +/- IFNα2b and GM-CSF - Immunological and clinical results. Proc Am Soc Clin Oncol 22:295 (#2850), 2003.

99. Shahied L, Alpaugh RK, Simmons H, Horak E, Somer R, Shaller C, Greer N, Lee J, Weiner LM. Cytotoxic potential of bispecific minibodies with monovalent or divalent tumor antigen binding properties. Proc Am Assoc Cancer Res 44:363 (#5782), 2003.

100. Adams GP, Robinson MK, Horak E, Heitner T, Garrson JL, Shaller CC, Tesfaye A, Simmons HH, Marks JD, **Weiner LM**. Anti-HER2/HER3 bispecific single-chain Fv molecules for cancer detection and therapy. Proc Am Assoc Cancer Res 44:363 (#5784), 2003.

101. Cheng JD, Valianou M, Simmons H, Jann P, Yuan QA, Russeva M, Marasco W, Cukierman E, Adams GP, **Weiner LM**. Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. Proc Am Assoc Cancer Res 45:85 (#377), 2004.

102. Tolcher AW, Mita M, Patnaik A, Rowinsky EK, Corey A, Fleming M, Fox NL, **Weiner LM**, Meropol NJ, Padavic K, Cohen R. A Phase I pharmacokinetic study of HGS-ETR1(TRM-1), a fully human monoclonal agonist-antibody to TRAIL R1, in patients with advanced solid tumors. Proc Am Soc Clin Oncol 23:210 (#3060), 2004.

103. Kirkwood JM, Lee S, Land S, Sander C, Mascari R, **Weiner LM**, Whiteside TL. E1696: Final analysis of the clinical and immunological results of a multicenter ECOG phase II trial of multi-epitope peptide vaccination for stage IV melanoma with MART-1 (27-35), gp100 (209-217, 210M), and tyrosinase (368-376, 370D) (MGT) +/- IFNα2b and GM-CSF. Proc Am Soc Clin Oncol 23:707 (#7502), 2004.

104. Cohen SJ, Palazzo I, Meropol NJ, Rogatko A, **Weiner LM**, Golemis E, Cheng JD, Alpaugh RK. Fibroblast activation protein and focal adhesion kinase: potential novel therapeutic targets in pancreatic adenocarcinoma. Proc Am Soc Clin Oncol 23:335 (#4094), 2004.

105. McDermott R, Meropol N, **Weiner LM**, Yeslow G, Hudes G. Phase I clinical and pharmacokinetic study of the farnesyl transferase inhibitor BMS-214662 administered intravenously for five consecutive days in patients with advanced malignancies. Proc Am Soc Clin Oncol 23:135 (#2033), 2004.

106. Konski A, Cheng JD, Goldberg M, Meropol NJ, Scott W, Haluszka O, Maurer A, Eisenberg D, **Weiner LM**. Percent SUV descrease may predict for disease-free survival in patients with esophageal carcinoma. ASCO GI Oncology Symposium, 2004.

107. Smirnov DA, Zweitzig DR, Foulk BW, Doyle GV, Pienta KJ, **Weiner LM**, Cohen SJ, Moreno JG, Connelly MC, Terstappen LWMM, O'Hara SM. Global gene expression profiling of circulating tumor cells. Proc Amer Assoc for Cancer Res (#5571), 2005.

108. Kaufman HL, Wang W, Manola J, DiPaola RS, Ko Y-J, Sweeney CJ, Whiteside T, Schlom J, Wilding G, **Weiner LM**. Phase II prime/boost vaccination using poxviruses expressing PSA in hormone dependent prostate cancer: Follow-up clinical results from the ECOG 7897. Proc Am Soc Clin Oncol 23(16S): 378s (#4501), 2005.

109. **Weiner LM**, Belldegrun A, Rowinsky E, Crawford J, Lockbaum P, Huang S, Arends R, Schwab G, Figlin R. Updated results from a dose and schedule study of Panitumumab (ABX-EGF) monotherapy, in patients with advanced solid malignancies. Proc Am Soc Clin Oncol 23(16S): 206s (#3059), 2005.

110. Haley M, Konski A, Li T, Cheng JD, Goldberg M, Maurer A, Haluszka O, Scott W, Meropol NJ, Cohen SJ, Freedman G, **Weiner LM**. The effect of diabetes in interpreting post-chemoradiation PET scans in patients with esophageal cancer. ASCO GI Onc Symp, 2006.

111. Tang Y, Alpaugh RK, Lou J, Litwin S, Shaller C, Adams GP, Marks JD, **Weiner LM**. High affinity promotes more effective ADCC by anti-HER2/neu monoclonal antibodies. Proc Amer Assoc for Cancer Res (#1172), 2006.

112. Binyamin L, Alpaugh RK, Borghaei H, Campbell KS, **Weiner LM**. Rituximab-mediated ADCC is augmented by concomitant interference with inhibitory self-recognition by human NK cells. Proc Amer Assoc for Cancer Res (#635), 2006.

113. Tang Y, Alpaugh RK, Lou J, Litwin S, Shaller C, Adams GP, Marks JD, **Weiner LM**. High affinity promotes more effective ADCC by anti-HER2/neu monoclonal antibodies. Proc Amer Soc Clin Oncol 24(18S): 109s (#2538), 2006.

114. Cheng JD, Cohen SJ, Stzempkowski-Brun B, Magalong K, Christiansen VJ, McKee PA, Alpaugh RK, Lee H, **Weiner LM**, Meropol NJ. Phase II pharmacodynamic study of the fibroblast activation protein inhibitor Val-boro-Pro in patients with metastatic colorectal cancer. Proc Amer Soc Clin Oncol 24(18S): 169s (#3594), 2006.

115. **Weiner LM.** Unconjugated antibodies for cancer therapy. 11th Conference on Cancer Therapy with Antibodies and Immunoconjugates, (#46): 2006.

116. Astsaturov A, Meropol NJ, Alpaugh RK, Cheng JD, Lewis NL, McLaughlin S, Rogatko A, Xu Z, **Weiner LM**, Cohen SJ. A randomized phase II study of bevacizumab alone or with docetaxel in patients with previously treated metastatic pancreatic adenocarcinoma. Proc Amer Soc Clin Oncol 25(18S):211s (#4556), 2007.

117. **Weiner LM**, Belldegrun A, Crawford J, Tolcher A, Yang B, Navale L, Amado RG, Figlin R. Safety, pharmacokinetics (PK) and activity of Panitumumab monotherapy in patients with advanced solid malignancies. ACCP (in press): 2007.

Naturally occurring antibodies devoid of light chains

C. Hamers-Casterman, T. Atarhouch, S. Muyldermans, G. Robinson*, C. Hamers, E. Bajyana Songa, N. Bendahman & R. Hamers†

Vrije Universiteit Brussel, Instituut voor Moleculaire Biologie, Paardenstraat 65, B-1640 Sint Genesius Rode, Belgium
* Department of Biochemistry, Trinity College, Dublin 2, Ireland

RANDOM association of VL and VH repertoires contributes considerably to antibody diversity[1]. The diversity and the affinity are then increased by hypermutation in B cells located in germinal centres[2]. Except in the case of 'heavy chain' disease[3], naturally occurring heavy-chain antibodies have not been described, although antigen binding has been demonstrated for separated heavy chains[4] or cloned VH domains[5]. Here we investigate the presence of considerable amounts of IgG-like material of M_r 100K in the serum of the camel (*Camelus dromedarius*)[6]. These molecules are composed of heavy-chain dimers and are devoid of light chains, but nevertheless have an extensive antigen-binding repertoire, a finding that calls into question the role of light chains in the camel. Camel heavy-chain IgGs lack CH1, which in one IgG class might be structurally replaced by an extended hinge. Heavy-chain IgGs are a feature of all camelids. These findings open new perspectives in the engineering of antibodies.

By a combination of affinity chromatography on protein A and protein G, three quantitatively important fractions corresponding to subclasses of IgG can be isolated from the serum of camels (Fig. 1*A*, lanes c–f).

One fraction (IgG_1) contains molecules of M_r 170K (Fig. 1*B*, lane 2), which upon reduction yield 50K heavy chains and large 30K light chains (Fig. 1*C*, lane 2). The two other immunoglobulin fractions contain molecules of ~100K (Fig. 1*B*, lanes 1 and 3), which upon reduction yield only heavy chains of, respectively, 46K (IgG_2 fraction binding only to proin A) (Fig. 1*C*, lane 3) and 43K (IgG_3 fraction binding to protein A and protein G) (Fig. 1*C*, lane 1). These two IgG classes appear to lack the light chain completely.

To exclude the possibility that the light chains were only weakly associated with the heavy chains and lost during our selective purification, whole serum was size-fractionated by gel filtration. Coomassie blue staining of unreduced fractions revealed the sequential elution of the 170K IgG_1 followed by the incompletely resolved isotypes IgG_2 and IgG_3 (90K) (Fig. 1*D*, upper inset). Immunostaining of the same fractions after reduction confirmed that the light chains were present only in the 50K heavy-chain-containing fractions (Fig. 1*D*, lower inset).

A comparative study of old world camelids (*Camelus bactrianus and Camelus dromedarius*) and new world camelids (*Lama pacos, Lama glama and Lama vicugna*) showed that heavy-chain immunoglobulins are abundant in the sera of all species examined (data not shown) and total up to 75 per cent of the molecules binding to protein A.

The abundance of heavy-chain immunoglobulins in the serum of camelids raises the question as to whether they bear an extensive antigen-binding repertoire. This question could be answered by examining the IgG_1, IgG_2 and IgG_3 fractions from the serum of camels (*Camelus dromedarius*) with a high antitrypanosome titre[7]. In radio-immunoprecipitation, purified fractions of IgG_1, IgG_2 and IgG_3 derived from infected camels were shown to bind a large number of antigens present in a [^{35}S]methionine-labelled trypanosome lysate (Fig. 2*a*), indicating an extensive repertoire complexity for the three IgG classes. Conversely, in blotting experiments, [^{35}S]methionine-labelled trypanosome lysate binds to SDS-PAGE-separated IgG_1, IgG_2

† To whom correspondence should be addressed.

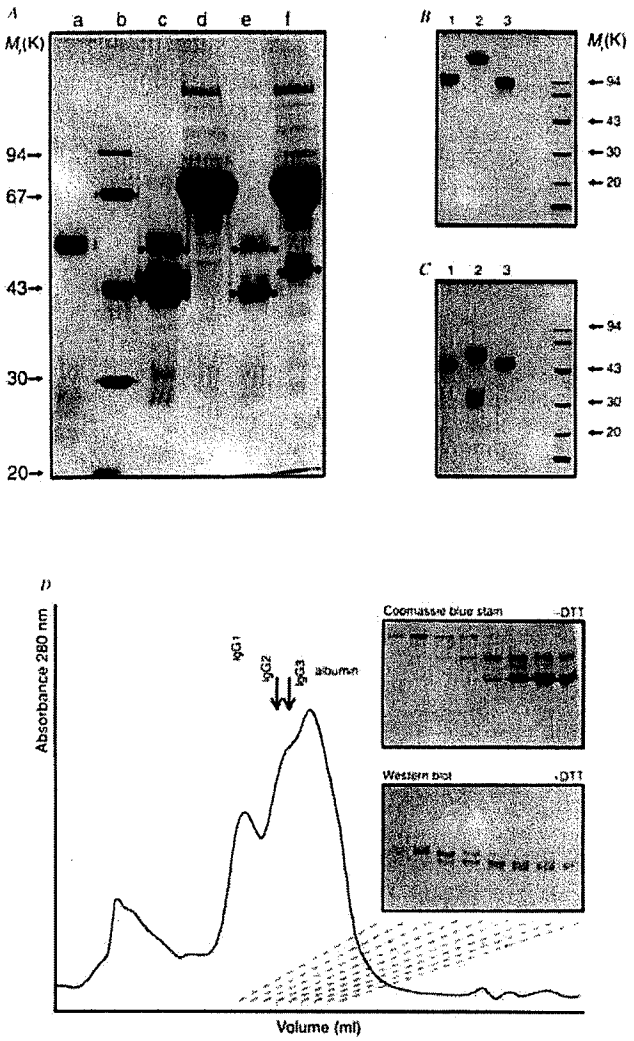


FIG. 1 Characterization and purification of camel IgG classes on protein A, protein G and gel filtration. *A*, The fraction of *C. dromedarius* serum adsorbed on protein A on reducing SDS-PAGE shows three heavy-chain components of 50, 46 and 43K (bands between dots), which are absent in the non-adsorbed fraction (lane d), and light-chain components of ~30K (lane c) that are considerably larger than rabbit light chain (lane a, rabbit IgG). The fractions adsorbed on protein G (lane e) lack the 46K heavy chain which remains in the non-adsorbed fraction (lane f). Lane b contains a size marker. *B* and *C*, By differential adsorption and elution on protein G and protein A, the IgG fractions containing 43K (lane 1), 46K (lane 3) and 50K (lanes 2) heavy chains were purified and analysed on SDS-PAGE in the absence (*B*) or presence (*C*) of dithiothreitol (DTT). *D*, Camel whole serum (0.1 ml) was fractionated by gel filtration on a Superdex-200 column using 150 mM NaCl, 50 mM sodium phosphate buffer, pH 7.0, as eluent. Affinity-purified IgG_2 and IgG_3 elute at the positions indicated by arrows. Fractions of interest were analysed further by SDS-PAGE with or without prior reduction. The protein contents as visualized by Coomassie blue (without reduction; upper inset) are compared with the immunoglobulins from the same fractions (after reduction with DTT, lower inset) as revealed by western blotting with a rabbit anti-camel IgG (lower inset).
METHODS. *C. dromedarius* serum (5 ml) is adsorbed onto a 5-ml protein G–Sepharose (Pharmacia) column and washed with 20 mM phosphate buffer, pH 7.0. Upon elution with 0.15 M NaCl, 0.58% acetic acid (pH 3.5), IgG_3 of 100K is eluted, which upon reduction yields heavy chains of 43K (lanes 1 in *B* and *C*). IgG_1 of 170K can subsequently be eluted with pH 2.7 buffer (0.1 M glycine-HCl). This fraction upon reduction yields a 50K heavy-chain and a broad light-chain band (lane 2 in *C*). The fraction not adsorbed on protein G is run on a 5-ml protein A-Sepharose column. After washing and elution with 0.15 M NaCl, 0.58% acetic acid (pH 4.5), IgG_2 of 100K is obtained, which consists solely of 46K heavy chains (lane 3 in *C*).

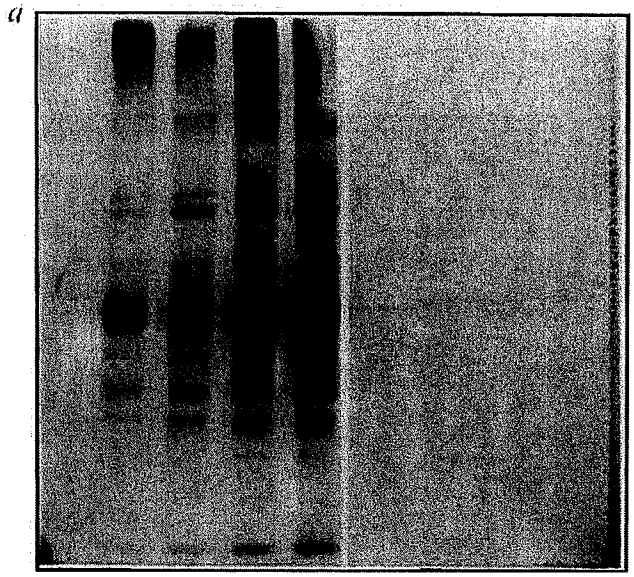


FIG. 2 Repertoire complexity and antigen-binding capacity of camel IgG_1, IgG_2 and IgG_3 analysed by *a*, radioimmunoprecipitation, or *b* and *c*, western blotting. *a*, Serum or purified IgG fractions from healthy or *Trypanosoma evansi*-infected *C. dromedarius* (CATT titre 1/160 (ref. 7)) were incubated with labelled trypanosome lysate, recovered with protein A–Sepharose and analysed by SDS–PAGE. The relative counts recovered are inscribed below each lane. No trypanosome proteins bind to the protein A or to the healthy camel immunoglobulins. *b*, 20 μg of IgG_1, IgG_2 and IgG_3 from healthy and trypanosome-infected animals were separated by SDS–PAGE without prior reduction or heating. The electroblotted proteins were incubated with the labelled trypanosome lysate. The IgG_2 shows a single antigen-binding component corresponding to the heavy-chain immunoglobulin, whereas the IgG_3 fraction appears in addition to contain two larger antigen-binding components barely detectable by Ponceau red staining (*c*). These could possibly be immunoglobulin classes copurified as immunocomplexes present in the serum of the infected animals.

METHODS. [^{35}S]methionine-labelled *Trypanosoma evansi* lysate (500,000 counts)[22] was incubated (4 °C, 1 h) with 10 μl serum or, 20 μg IgG_1, IgG_2 or IgG_3 in 200 μl of 0.4 M NaCl, 10 mM EDTA, 10 mM Tris, pH 8.3, containing 0.1 M *N*-α-tosyl-L-lysine chloromethyl ketone (TLCK). Protein A–Sepharose (10 mg) suspended in 200 μl of the same buffer was added (4 °C, 1 h). After washing and centrifugation, each pellet was resuspended in 75 μl SDS–PAGE sample solution containing DTT and heated for 3 min at 100 °C. After centrifugation, 5 μl supernatant was saved for radioactivity counting and the remainder analysed by SDS–PAGE and fluorography. The nitrocellulose filter of the western blot of purified fractions IgG_1, IgG_2 and IgG_3 was stained with Ponceau red (*c*) or incubated with 1% ovalbumin in TST buffer (Tris 10 mM, NaCl 150 mM, Tween 0.05%) (*b*). The membrane was extensively washed with TST buffer and incubated for 2 h with ^{35}S-labelled trypanosome antigen. To avoid nonspecific binding, labelled trypanosome antigen lysate was filtered (45 μm) and incubated with healthy camel immunoglobulin and ovalbumin adsorbed on a nitrocellulose membrane.

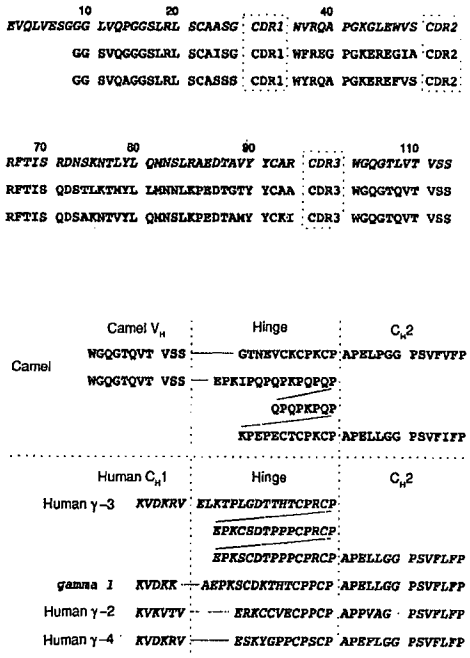


FIG. 3 Amino-acid sequences of the VH framework, and hinge/CH2 of *Camelus dromedarius* heavy-chain immunoglobulins, compared to human (italic) VH framework (subgroup III) and hinges of human IgG[14].

METHODS. Total RNA was isolated from a dromedary spleen[23]. mRNA was purified with oligo(T) paramagnetic beads (PolyATract, Promega). 1 μg mRNA was used for preparing double-stranded cDNA[23] after an oligo(dT) priming using enzymes from Boehringer Mannheim. 5 ng cDNA was amplified by PCR in a 100-μl reaction mixture (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) gelatine, 200 μM of each dNTP). 25 pmol of each oligonucleotide of mouse VH[24], containing an *Xho*I site, and 5′-CGCCAT-CAAGGTACCAGTTGA-3′ were used as primers. The 3′-end primer was deduced from partial sequences corresponding to γ-chain amino acids 296 to 288 (T. Atarhouch, C. H.-C. and G.R., unpublished results) in which one mismatch was introduced to create a *Kpn*I restriction site. After a round of denaturing annealing (94°C for 5 min and 54°C for 5 min), 2 U of *Taq* DNA polymerase were added to the reaction mixture before 35 cycles of amplification[5]. PCR products were purified by phenol–chloroform extraction followed by HPLC (Genpak-fax column, Waters) and finally by MERMAID (BIO101). After purification, amplified cDNA was digested with *Xho*I and *Kpn*I, and ligated into pBluescript. Clones were sequenced by dideoxy chain termination[25]. Sequences were then translated so that they could be assigned to well defined domains of the immunoglobulin molecule[14].

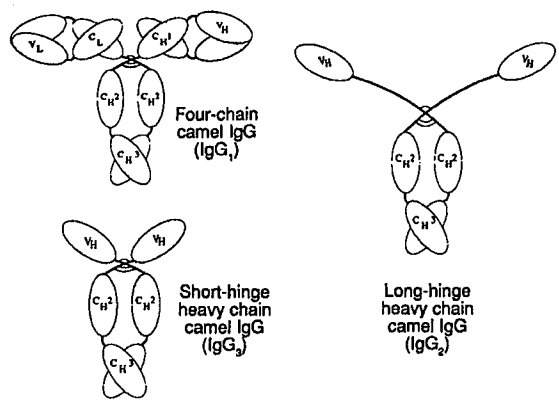


FIG. 4 Schematic representation of the structural organization of the camel immunoglobulins (adapted from ref. 26). On the basis of size, the IgG_1 fraction probably has the normal antibody assembly of two light and two heavy chains. IgG_3 would have a hinge comparable in size to the human IgG_1, IgG_2 and IgG_4. The two antigen-binding sites are much closer to each other as camel IgG lacks the CH1 domain. In camel IgG_2 the long hinge, which is formed of Pro-X repeats (X=Glu, Gln or Lys), probably adopts a rigid structure[19,20]. This long hinge could therefore substitute for the CH1 domain and bring the two antigen-binding sites of IgG_2 into normal positions.

and IgG_3 obtained from infected animals (Fig. 2*b*, *c*). These findings indicate that the heavy chains alone can generate an extensive repertoire and question the obligatory contribution of the light chain to the useful antibody repertoire in the camelids.

The camelid γ2 and γ3 chains are considerably shorter than the normal mammalian γ or camel γ1 chains. This would suggest that, as in the case of 'heavy chain' disease[3], deletions have occurred in the CH1 protein domain[8,9]. To address this question, complementary DNA was synthesized from camel spleen messenger RNA and the sequences between the 5′ end of the VH and the CH2 were amplified by polymerase chain reaction (PCR), and cloned. Seventeen clones presenting a different VH sequence were isolated and sequenced. Their most striking feature was a complete lack of the CH1 domain, the last framework (FR4) residues of the VH region being immediately followed by the hinge (Fig. 3, lower part). The absence of the CH1 domain clarifies two important dilemmas. First, immunoglobulin heavy chains are normally not secreted unless the heavy-chain chaperoning protein or BIP (ref. 10) has been replaced by the L chain[11], or alternatively the CH1 domain has been deleted[3,8,9]. Secondly, isolated heavy chains from mammalian immunoglobulins tend to aggregate, but are only solubilized by light chains[8,12] which bind to the CH1 and the VH domains[13].

Fourteen of the seventeen clones were characterized by a short hinge sequence with a length equal to that of human IgG_2 and IgG_4 (ref. 14) (Fig. 3). The other three had a long hinge sequence containing the 'EPK' hinge motif found in human IgG_1 and IgG_3 (ref. 14). They possess the CH2 'APELL/P' motif that is also found in human IgG_1 and IgG_3, and which is associated with mammary transport of bovine IgG_1 (ref. 15). On the basis of their molecular weights, we expect the 'short-hinge' clones to correspond to IgG_3 and the 'long-hinge' clones to IgG_2.

In the short hinge-containing antibody, the extreme distance between the extremities of the VH regions will be of the order of 80 Å, corresponding to twice the size of a single domain of 40 Å (2 × VH)[16]. This could be a severe limitation for agglutination, crosslinking or complement fixation[17,18]. In the long-hinge-containing immunoglobulin, the absence of CH1 might be compensated by the extremely long hinge itself, composed of a 12-fold repeat of the sequence Pro-X (where X is Gln, Glu or Lys) (Figs 3 and 4). NMR (ref. 19) and molecular modelling[20] of Pro-X repeats present in the TonB protein of *Escherichia coli* (in which X is Glu or Lys) and of the membrane procyclin of trypanosomes (X is Asp or Glu) indicate that these repeated sequences function as rigid rod-like spacers with a diameter of 8 Å and a rise of 2.9 Å per residue. Assuming the same geometry, the long hinge would be 70 Å, which compensates for the absence of the CH1 domain.

The binding site of heavy-chain antibodies cannot form the pocket resulting from adjoining light and heavy chain V regions, and the residues of VH that normally interact with VL will be exposed to solvent[3,5,13]. Leucine at position 45 is conserved in 98% of human and murine VH sequences[14] and is crucial in the VH–VL association[13]; it can be replaced by an arginine (Fig. 3, top section). This substitution is in accordance with both the lost contact with a VL domain and an increased solubility.

Unlike myeloma heavy chains, which result mainly from CH1 deletion in a single antibody-producing cell[21], the camelid heavy-chain antibodies have emerged in a normal immunological environment and will probably have undergone the selective refinement in specificity and affinity that accompanies B-cell maturation[1,2]. The obtention of camelid heavy-chain antibodies could therefore be an invaluable asset in the development and engineering of soluble VH domains[5] or of new immunological molecules for diagnostic, therapeutic and biochemical purposes. □

Received 20 January; accepted 22 March 1993.

1. Tonegawa, S. *Nature* **302,** 575–581 (1983).
2. Jacob, J., Kelsoe, G., Rajewski, K. & Weiss, U. *Nature* **354,** 389–392 (1991).
3. Fleischman, J. B., Pain, R. H. & Porter, R. R. *Arch. Biochem. Biophys.* Suppl. **1,** 174–180 (1962).
4. Utsumi, S. & Karush, F. *Biochemistry* **3,** 1329–1338 (1964).
5. Ward, E. S., Güssow, D., Griffiths, A.D., Jones, P. T. & Winter, G. *Nature* **341,** 544–546 (1989).
6. Ungar-Waron, H., Eliase, E., Gluckman, A. & Trainin, Z. *Isr. J. vet. Med.* **43,** 198–203 (1987).
7. Bajyana Songa, E. & Hamers, R. *Ann. Soc. Belge Méd. Trop.* **68,** 233–240 (1988).
8. Seligmann, M., Mihaesco, E., Preud'homme, J.-L., Danon, F. & Brouet, J.-C. *Immun. Rev.* **48,** 145–167 (1979).
9. Traunecker, A., Schneider, J., Kiefer, H. & Karjalaien, K. *Nature* **339,** 68–70 (1989).
10. Henderschot, L. M., Bole, D., Köhler, G. & Kearney, J. F. *J. Cell Biol.* **104,** 761–767 (1987).
11. Henderschot, L. M. *J. Cell Biol.* **111,** 829–837 (1990).
12. Roholt, O., Onoue, K. & Pressman, D. *Proc. natn. Acad. Sci. U.S.A.* **51,** 173–178 (1964).
13. Chothia, C., Novotny, J., Bruccoleri, R. & Karplus, M. *J. molec. Biol.* **186,** 651–663 (1985).
14. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M. & Gottesman, K. S. *Sequences of Proteins of Immunological Interest* 511 (US Dept of Health and Human Services, US Public Health Service, NIH, Bethesda, 1987).
15. Jackson, T., Morris, B. A. & Sanders, P. G. *Molec. Immun.* **29,** 667–676 (1992).
16. Poljak, R. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **70,** 3305–3310 (1973).
17. Dangl, J. L., *et al. EMBO J.* **7,** 1989–1994 (1988).
18. Schneider, W. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 2509–2513 (1988).
19. Evans, J. S. *et al. FEBS Lett.* **208,** 211–216 (1986).
20. Rodlti, I. *et al. J. Cell Biol.* **108,** 737–746 (1989).
21. Dunnick, W., Rabbits, T. H. & Milstein, C. *Nucleic Acids Res.* **8,** 1475–1484 (1980).
22. Bülow, R., Nonnengässer, C. & Overath, P. *Molec. Biochem. Parasitol.* **32,** 85–92 (1989).
23. Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual* 2nd edn (Cold Spring Harbor Laboratory Press, New York, 1989).
24. Sastry, L. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 5728–5732 (1989).
25. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
26. Klein, J. *Immunology* (Blackwell Scientific, London, 1990).

ACKNOWLEDGEMENTS. We thank A. Dakkak, P. Sayer and R. Dedeken for assistance in obtaining samples of camelid sera, W. Verheulpen for preparing the figures, and L. Eeckhaudt for typing the manuscript. This research was supported by the Biotechnology programme of the Flemisch regional Government and by the ECC programmes 'Science and Technology for Development.

Expression of a Dromedary Heavy Chain-Only Antibody and B Cell Development in the Mouse[1]

Xiangang Zou,* Jennifer A. Smith,* Viet Khong Nguyen,[2†] Liming Ren,[3*] Kattie Luyten,* Serge Muyldermans,[†] and Marianne Brüggemann[4*]

In mature B cells of mice and most mammals, cellular release of single H chain Abs without L chains is prevented by H chain association with Ig-specific chaperons in the endoplasmic reticulum. In precursor B cells, however, surface expression of μ-H chain in the absence of surrogate and conventional L chain has been identified. Despite this, Ag-specific single H chain Ig repertoires, using μ-, γ-, ϵ-, or α-H chains found in conventional Abs, are not produced. Moreover, removal of *H* chain or, separately, *L* chain (κ/λ) locus core sequences by gene targeting has prevented B cell development. In contrast, H chain-only Abs are produced abundantly in Camelidae as H2 IgG without the C_H1 domain. To test whether H chain Abs can be produced in mice, and to investigate how their expression affects B cell development, we introduced a rearranged dromedary γ2a H chain into the mouse germline. The dromedary transgene was expressed as a naturally occurring Ag-specific disulphide-linked homodimer, which showed that B cell development can be instigated by expression of single H chains without L chains. Lymphocyte development and B cell proliferation was accomplished despite the absence of L chain from the BCR complex. Endogenous Ig could not be detected, although V(D)J recombination and IgH/L transcription was unaltered. Furthermore, crossing the dromedary H chain mice with mice devoid of all *C* genes demonstrated without a doubt that a H chain-only Ab can facilitate B cell development independent of endogenous Ig expression, such as μ- or δ-H chain, at early developmental stages. *The Journal of Immunology,* **2005, 175: 3769–3779.**

In the conventional mouse and human immune system, B cell development is initiated by VDJ recombination and surface IgM expression (Ref. 1, and refs. therein). At the pre-B cell stage, the associated surrogate L chain is replaced with a κ- or λ-L chain, and this initiates the process of Ab maturation, which is accompanied by cellular migration and class switching. At this stage, mature B cells undergo further selection and affinity maturation and can differentiate into Ab-secreting plasma cells or memory cells bearing other isotypes (IgG, IgA, or IgE). Developmental progression is blocked at the pre-B-I cell stage when H chain expression is prevented, although H and/or L chain transcripts may be found (2, 3). Likewise, silencing of both κ- and λ-*L* chain loci blocks B cell development, but at the somewhat later pre-B-II stage, which allows normal development up to pre-BCR expression (4). With the lack of L chain, μ-H chain is retained in the cytoplasm of immature bone marrow B cells, and their further development, with subsequent migration and colonization of the spleen, is prevented (4). Targeted modification of the *IgH* locus has permitted expression of truncated Ig polypeptides (5), and introduction of Ig transgenes consisting of shorter chains or removed domains has allowed single chain expression (6–8). Recently, it has also been shown that entire μ-H chains in association with the Igα coreceptor, but lacking surrogate or conventional L chain, can be expressed on the cell surface of pre-B cells. Single μ-H chain expression may induce differentiation signals and allow developmental progression possibly up to the immature B cell stage (9–11). Nevertheless, there are no examples where individual H chain polypeptides, on the surface or released from the cell without associated L chain, facilitate B cell differentiation to the mature and specialized stage leading to Ab repertoire formation in the mouse.

Abs, consisting of multiple units of paired H and L chains (12), emerged early in vertebrate evolution, and their presence is demonstrated in all of the jawed vertebrates studied to date (13). In addition to these conventional heteromeric Abs, sera of camelids (suborder Tylopoda, which includes camels, dromedaries, and llamas) contain a major type of Ig composed solely of paired H chains (14). Homodimeric H chain Abs in camelids lack the first C domain (C_H1) but harbor an intact variable domain (V_HH) encoded by different, clearly distinguishable, *V* genes (15). Using structural analysis, it has been concluded that it is impossible for a V_HH to pair with a normal V_L because the V_L-interacting side of the domain is reshaped by the hydrophilic V_HH hallmark amino acids and the long CDR3, which folds over this region (16). H chain Abs are absent in other mammals except in pathological cases, known as heavy chain disease, where parts of the V_H domain and/or C_H1 exon have been removed (17). Interestingly, H chain Abs are also present in some primitive fish; e.g., the new Ag receptor in the nurse shark and the specialized H chain (COS5) in ratfish (18, 19). Evolutionary analysis showed that their genes emerged and evolved independently, whereas *H* chain genes in camelids evolved from pre-existing genes used for conventional heteromeric Abs (20).

In camelids, the problem of developmental progression when single H chains are expressed may be circumvented because of

*Protein Technologies Laboratory, The Babraham Institute, Babraham, Cambridge, United Kingdom; and †Laboratory of Cellular and Molecular Immunology, Department of Molecular and Cellular Interactions, Vlaams Interuniversitair Instituut voor Biotechnologie, Vrije Universiteit Brussel, Brussels, Belgium

Received for publication November 15, 2004. Accepted for publication June 13, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the Babraham Institute and the Biotechnology and Biological Sciences Research Council.

[2] Current address: Central Vietnam Veterinary Institute, Agricultural and Rural Development Ministry, Milestone 4, Avenue 2/4, DongDe, NhaTrang, Vietnam.

[3] Current address: State Key Laboratory for Agrobiotechnology, College of Biological Science, China Agricultural University, Beijing 100094, China.

[4] Address correspondence and reprint requests to Dr. Marianne Brüggemann, The Babraham Institute, Babraham, Cambridge CB2 4AT, United Kingdom. E-mail address: marianne.bruggemann@bbsrc.ac.uk

0022-1767/05/$02.00

structural differences in particular *V* and γ-*C* genes. As in conventional Ab production, the *H* chain gene of a H chain Ab is obtained after DNA rearrangements when specific V_HH germline genes, located within the V_H gene cluster, are assembled with commonly used *D* and J_H segments to code for the V_HH-domain (21). Genomic and cDNA analyses have revealed five functional dromedary γ genes, three of which (*γ2a*, *γ2c*, and *γ3*) are always used to form H chain-only isotypes, whereas two separate genes, *γ1a* and *γ1b*, are used for the production of heterodimeric IgG isotypes (22). Only a very low yield of H chain-only Ab transcripts (identified by their particular *V* genes) spliced to Cμ (V_HHDJ_H-Cμ) have been identified from dromedary spleen (23). Serum IgM devoid of L chains has not been found, and staining of camelid B cells for IgG H chain-only Ab is not yet possible due to a lack of specific Abs. These observations indicate that the IgM stage of H chain Abs may be transient and that the conventional differentiation events initiated by IgM expression may be circumvented.

The lack of C_H1 in H chain Abs is most likely to be the crucial factor in allowing their release from cells in the absence of L chains. Although H chain *C* region genes encode the first exon, it is spliced out during mRNA maturation, probably due to a point mutation at the canonical splicing donor site (24, 25). It has been established that the C_H1 domain participates actively in the regulation of assembly and secretion of conventional H2L2 Abs. The nascent-translated H chain polypeptide associates noncovalently with the H chain binding protein (BiP[5] or grp78) via BiP association sites in C_H1 (26). The BiP/H chain complex is retained in the endoplasmic reticulum by virtue of the KDEL sequence at the carboxy terminus of BiP (27), and an H chain with a C_H1 domain is not secreted unless BiP is displaced by the L chain (26, 28, 29).

It is possible that H chain-only Abs have been selected and maintained in Tylopoda for their complementary function in recognizing unusual epitopes, such as clefts on the Ag surface that are normally less antigenic for conventional Abs (30). So it could be argued that H chain Abs are maintained because they fulfill a complementary function in their humoral immune response. In the peripheral blood of all camelids, H chain Abs of different isotypes contribute to the immune response; they undergo Ag-mediated selection and affinity maturation, and their V_HHDJ_H domains are subjected to extensive somatic hypermutation (21, 22).

Our experiments focus on two essential questions. Can a dromedary H chain gene be processed and correctly expressed in the mouse? A feasibility study to evaluate the prospect of generating therapeutic camel H chain Abs in appropriately engineered mice is crucial to allow the future creation of H chain Ab repertoires. And, are conventional developmental processes capable of permitting B cell maturation without L chain association? This is important because L chain-dependent development or coexpression may impede the desired production of high-affinity dimeric H chains with their prospective use as enzyme inhibitors.

In this article, we show that H chain-only Ab expression of a rearranged dromedary *γ2a* gene in transgenic mice initiates B cell development without involvement of L chain or production of endogenous IgM. Allelic feedback signals are operative and seem to secure isotype exclusion by preventing expression but not V(D)J rearrangement and transcription of endogenous H and L chain genes. Multimeric IgG2a H chains are efficiently secreted, and their assembly on the cell surface allows Ag recognition. Expression of H chain Abs, with their particular configuration of the V_HH gene, their C_H1 exon removed by splicing, and lack of L chain, does not appear to be regulated by the presence of a classical BCR complex.

[5] Abbreviations used in this paper: BiP, H chain binding protein; BIO, biotin; HEL, hen egg lysozyme; KO, knockout.

Materials and Methods

Derivation of mice

The dromedary V_HH-*Cγ2aTM* H chain Ab gene on an 11.7-kb *NotI-SalI* fragment (31) was purified using a DNA purification kit (no. 28304; Qiagen). For the derivation of transgenic mice, DNA was microinjected into the male pronucleus of F_1 embryos (CBA × C57BL/6) according to standard methods (32), and several founders were produced. Transgenic animals were identified by PCR using dromedary Cγ2a-specific oligonucleotides: C_HH1 forward, 5′-GCACCTCGACCGGAAAGACCTTCATCTCC-3′; and hinge reverse, 5′-GGGACACGTGCATTCTGGTTCAGG-3′, which produced a 485-bp fragment (data not shown). Two animals, judged by PCR and Southern hybridization to be high (cam1) and low (cam2) copy number founders, were bred to homozygosity with μMT mice (33) and CΔ mice, in which all C genes had been removed by Cre-*lox*P-mediated deletion (3). Southern blot analysis (34) was conducted on *Kpn*I digests of tail DNA hybridized with a 1.4-kb *NotI-Bst*EII V_HH-gene probe (31).

Nucleic acid preparations and PCR

For transcriptional analysis of transgene expression, mRNA was prepared from bone marrow and spleen cells using the RNeasy Mini Kit (no. 74104; Qiagen), and cDNA was prepared with the Omniscript RT Kit (no. 205111; Qiagen) according to manufacturer's instructions. RT-PCR primers for identifying transgenic expression (see Fig. 2) were as follows: V3FR1B (V_HH), 5′-GAGGTGCAGCTGGTGGCGTCTGGAGGAGG-3; G2AH1F (H, hinge), 5′-GGGACACGTGCATTCTGGTTCA-3′; H (hinge) forward, 5′-CAACCAAAACCTGAACCAGAATGC-3′; C_HH2 reverse, 5′-G CACCTCAACGCCATCAATG-3′; C_HH3 forward, 5′-CAAGGACACCG TGAGCATAACCT-3′; 3′C_HH3 reverse (downstream of the stop codon), 5′-TGCCGGGGTGAGGCTCATTTA-3′; and M2 reverse, 5′-GCC CGATCATGTTTCTGTAGTCTG-3′. Lamin B1 served as a control to normalize the DNA concentration and also allowed discrimination of cDNA and genomic PCR products (3). PCR conditions were 93°C for 2 min followed by 32 cycles of 30 s at 93°C, 45 s at 58°C, and 90 s at 72°C, followed by 10 min at 72°C to complete the reaction.

For the analysis of D-J_H, V_H-D-J_H, and $V_κ$-$J_κ$-$C_κ$ rearrangement and transcription, as well as surrogate L chain transcription, RNA was prepared from bone marrow and spleen cells using Tri Reagent (Sigma-Aldrich), and cDNA was prepared using the Omniscript RT kit as above. Genomic DNA was prepared by lysis with proteinase K at 55°C, phenol/chloroform/isoamyl alcohol (25/24/1; Sigma-Aldrich) extraction and ethanol precipitation. Combinations of the following oligonucleotides were used: for the H chain, a 1/1 mixture of DF (5′- GCATGTCTCAAAGCACAATG-3′) and DQ52 (5′-ACCCTGGA CACAGGAAACAC-3′); VJ558L (5′-ATGGGATGGAGCTGGATCTT-3′) for DNA; and a 1/1 mixture of VJ558L and VJ558CL (5′-ATGGAATG GAGCTGGGTCTT-3′) for cDNA and V7183 (5′-ATGAACTTCGGGCT CAGCTT-3′) forward primers in combination with J_H1-4 reverse primer (5′-GAGACDGTGASHRDRGTBCCTKSRCC-3′) (3); for the L chain $V_κ$ forward (5′-GGCTGCAGSTTCAGTGGCAGTGGRTCWGGRAC-3′) (35); universal Jκ (5′-GTTTKATTTCCARYYTKGTSCC-3′) and Cκ reverse (5′-GCTCATGCTGTAGGTGCTGTCTTTGCTGTC-3′); and for surrogate L chain λ5 forward (5′-AGTTCTCCTCCTGCTGCTGCTGT-3′); λ5 reverse (5′-TACCTTCCAGTCCACCACCAAAG-3′); VpreB forward (5′-TGGT CAGGKCCCAGGAGCAGTGG −3′); and VpreB reverse (5′-CCGGAGC CCCACRGCRCAGTAAT-3′). PCR conditions were 30–35 cycles of 93°C for 20 s, 62–64°C for 30–35 s, and 72°C for 40–50 s, followed by 10 min at 72°C to complete the reaction, and RT-PCR conditions were 2 min at 94°C followed by 32 or 33 cycles of 93°C for 20 s, 62–64°C (70°C for λ5 and Vκ-Cκ, 50°C for J558-J_H1-4 RT-PCR) for 30–45 s, and 72°C for 50–60 s, followed by 10 min at 72°C to complete the reaction. The hybridization probe for the V558-J_H PCR was a 450-bp *Eco*R1 fragment from a cloned J558 family member, kindly supplied by A. Wood (Babraham Institute).

ELISA and Western blot analyses

Serum Abs were identified by ELISA (36) on Falcon plates (no. 353911; BD Biosciences) coated with 10 μg/ml goat anti-llama IgG (no. A160-100A; Bethyl Laboratories). Bound Abs were detected either with HRP-conjugated goat anti-llama IgG (no. A160-100P; Bethyl Laboratories) or biotin (BIO)-conjugated rat anti-mouse κ L chain (no. 04-6640; Zymed Laboratories) or BIO-conjugated anti-mouse $λ_{1,2,3}$ L chain (BD Pharmingen) developed with streptavidin-biotinylated HRP (no. RPN1051; Amersham Biosciences).

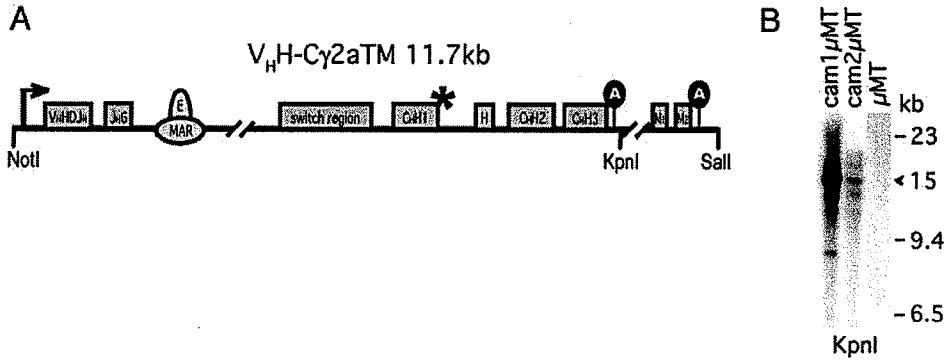


FIGURE 1. Integration of the dromedary *IgG2a* gene in the mouse germline. *A*, The dromedary H chain gene on an 11.7-kb *NotI-SalI* fragment comprises a rearranged V_HHDJ_H gene, intervening sequences, including matrix attachment region (MAR), and Eμ enhancer (E), followed by the switch region and the genomic region of the dromedary *Cγ2a* gene (exons C_HH1, hinge (H), C_HH2, and C_HH3), including the membrane exons (M1 and M2) (31). The mutation in the donor splice site (*), the poly(A) sites (*A*), and the *KpnI* site, for mapping analysis, are indicated. *B*, Southern blot analysis of tail DNA from cam1μMT and cam2μMT mice. DNA was digested with *KpnI*, blotted, and hybridized with a 1.4-kb V_HH-specific probe (see *Materials and Methods*) to identify transgene integration and copy number. μMT DNA served as a control. λ*HindIII* was used as a size marker.

Goat anti-llama IgG (1 mg/ml) was coupled to CNBr-activated Sepharose 4B (no. 17-0430-01; Amersham Biosciences) in 0.1 M sodium carbonate buffer (pH 8.5) and stirred gently overnight at 4°C. Coupled Sepharose was left in 1 M glycine for 2 h and then washed and stored in PBS 0.5% sodium azide. Serum (20 μl) was incubated with ~20 μl of anti-llama IgG Sepharose overnight, and unbound proteins were removed by washing with PBS. For Western blot analysis, proteins were separated on precast 4–15% Tris-HCl Ready-Gels (no. 161-1104; Bio-Rad) and transferred to nitrocellulose membranes as described previously (31). Filters were incubated with HRP-conjugated goat anti-llama IgG, or BIO-conjugated rat anti-mouse κ L chain or anti-mouse $\lambda_{1,2,3}$ L chain, followed by incubation with streptavidin-biotinylated HRP as described above, and chemiluminescent substrate (SuperSignal West Pico, no. 34080; Pierce) was used for detection according to the manufacturer's protocol. Restore Western blot stripping buffer was used in some experiments (no. 21059; Pierce). The m.w. marker was All Blue Standards (no. 161-0373; Bio-Rad).

Flow cytometry analyses

Bone marrow and spleen cell suspensions were prepared from cam1μMT, cam2μMT, μMT, cam1, cam2, cam2CΔ, $RAG2^{-/-}$, and normal F_1 mice. Cells were stained in combination with allophycocyanin-conjugated anti-mouse CD45R (B220) (no. 01129A; BD Pharmingen), FITC-conjugated anti-mouse IgM (no. 04-6811; Zymed Laboratories), PE-conjugated anti-mouse c-*kit* (CD117) (no. 09995B; BD Pharmingen) and/or BIO-conjugated anti-mouse CD43 (no. 01602D; BD Pharmingen), FITC-conjugated anti-mouse IgM, PE-conjugated anti-mouse Igκ (no. 559940; BD Pharmingen), FITC-conjugated anti-mouse Igλ (no. 021174D; BD Pharmingen), FITC-conjugated anti-mouse CD21/35 (no. 553818; BD Pharmingen), and BIO-conjugated hen egg lysozyme (HEL) (31). Reactions with BIO-conjugated Abs were subsequently incubated with Tri-color-conjugated streptavidin (no. SA1006; Caltag Laboratories). Cytoplasmic staining was conducted using a fix and perm cell permeabilization kit with reduced background formulation (GAS-004; Caltag Laboratories) according to the manufacturer's instructions. Cells were analyzed on a FACSCalibur (BD Biosciences), and CellQuest (BD Biosciences) was used for data analysis. A FACSAria (BD Biosciences) was used for sorting $B220^+$ and $B220^-$ lymphocyte populations at up to 12,000 cells per second, which resulted in a purity >95%.

Results

Integration of a dromedary H chain gene construct in the mouse germline

The H chain Ab gene, V_HH-*Cγ2aTM*, has been constructed using a rearranged dromedary V_HHDJ_H gene with specificity for HEL and a dromedary *Cγ2a* gene in germline configuration, including the transmembrane exons (31). Figure 1*A* shows the 11.7-kb *NotI-SalI* fragment, which was microinjected into fertilized mouse oocytes. From the animals born, two were selected (cam1 and cam2, identified by PCR) for further breeding. These represented high copy number and low copy number transgenic founder mice. The animals were crossed with μMT mice in the C57BL/6 background (33), which resulted in $cam^{+/-}\mu MT^{-/-}$ and $\mu MT^{-/-}$ animals, which were used for detailed analyses. In Southern blotting, *KpnI* digests showed several bands containing the transgene (Fig. 1*B*) with a predominant ~15-kb fragment, suggesting multiple and tandem integration as the construct harbors a ~7.4-kb *NotI-KpnI* fragment. Comparison of signal intensities estimated that the cam1μMT mice have the transgene integrated at a high copy number (>40 tandem copies), whereas cam2μMT is a low copy number (≥2 copies) line.

H chain transcripts are correctly spliced

An important question was whether the introduced H chain gene would be transcribed and whether the resulting product would match the transcripts found in Camelids. To investigate possible mRNA splice products, we used RT-PCR and sets of oligonucleotides that would reveal the exon usage (Fig. 2). Employment of V forward and hinge reverse oligonucleotides revealed a product of 500 bp from cam1 and cam2 mice (Fig. 2*A*), which corresponded to V_HHDJ_H-hinge splice products lacking the C_H1. Inclusion of the C_H1 exon would have increased the size to ~800 bp (31). The hinge region is followed by C_HH2 (Fig. 2*B*). Transcripts that allow Ig secretion (Fig. 2*C*) and surface expression (Fig. 2*E*, *top bands*) were found, both in bone marrow and spleen cells, as has been described for μ mRNA products in mouse B cells (37). Figure 2, *D* and *F*, illustrate the exon usage of the PCR products, which have been confirmed by cloning and sequencing (supplemental Table I).[6] Simultaneous amplification of lamin B1 (Fig. 2*E*, *bottom bands*) served as a semiquantitative reference and suggested that secretory and membrane transcript levels are higher in spleen and bone marrow cells from cam2 mice. In cam1 mice, we found little transmembrane product and a diminished intensity of the amplification bands. This implies low transcription levels of the dromedary transgene in cam1 mice, which may be due to the integration site, e.g., in a transcriptionally silent region, and is reaffirmed by ELISA and flow cytometry analysis shown below. Despite differences in expression levels, the results show correctly spliced H chain products, without C_H1, in bone marrow and spleen, which

[6] The online version of this article contains supplemental material.

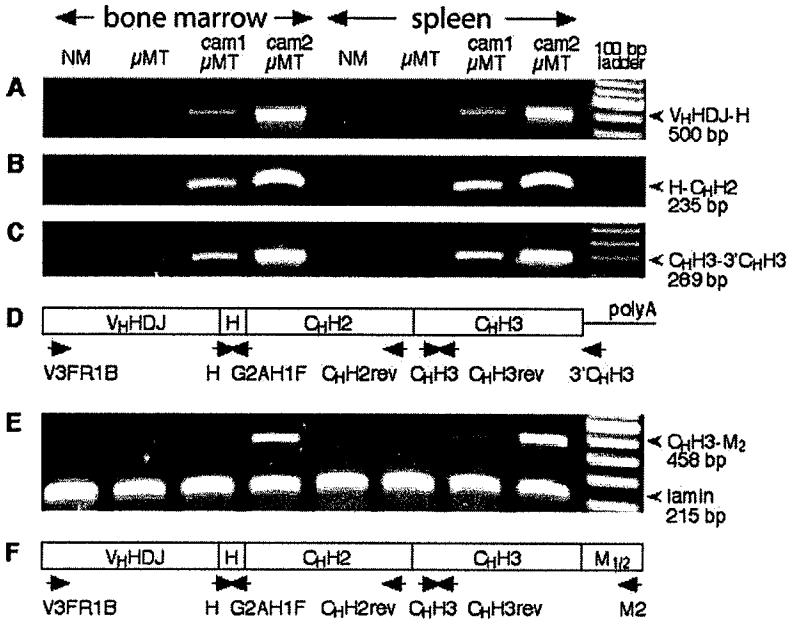


FIGURE 2. Transcription of dromedary H chain in bone marrow and spleen. RT-PCR analysis was conducted using oligonucleotides priming in the following regions: *A*, V_HH and hinge (H); *B*, H and C_HH2; *C*, C_HH3 and 3′ of C_HH3 stop codon; *E*, C_HH3 and membrane exon 2 (M2) and lamin, as control conducted in parallel, to verify matching cDNA concentrations. *D* and *F*, Maps established from the product sizes. These show that the C_HH1 exon is omitted and that the correctly transcribed dromedary H chain consists of V_H-HDJ-H-C_HH2-C_HH3-M1/2. The secreted (*D*) and membrane (*F*) forms are found in both bone marrow and spleen cell populations. As a size marker, a 100-bp ladder was used. In addition, the exact size of each band was established by DNA sequencing (supplemental Table I).

implies that the introduced dromedary *H* chain gene is faithfully expressed in both secreted and transmembrane form.

Multimeric Ig is secreted in serum

To analyze secretion of dromedary H chain Ig, we captured serum Abs from the camμMT mice in a sandwich ELISA using goat anti-llama IgG for detection. Figure 3 illustrates strong Ab binding of two representative cam2μMT mice (termed a and b), the low copy transgenic line, with good detection of up to 1/1000 dilution. The high copy line, cam1μMT, had a low Ab titer (detectable only in 1/3 (data not shown) and 1/10 serum dilutions), whereas background binding was obtained when using μMT and normal mouse serum. Because binding to anti-llama IgG did not reveal the assembly of the secreted dromedary IgG2a, we further tested serum Abs for the presence of L chain. None of the cam1μMT, cam2μMT, and μMT sera showed binding to anti-mouse Igκ or anti-mouse Igλ L chain; however, normal mouse serum revealed some cross-reactivity, in that weak binding to anti-llama Ig could be detected with anti-Igκ.

To assess the assembly and m.w. of the secreted H chain Ig, we conducted Western blot analyses. To overcome a high background,

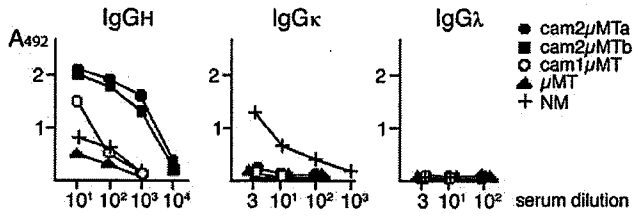


FIGURE 3. Expression of H chain-only Abs in serum of cam mice. H chain Abs (IgG_H) were identified in ELISA by coating and detection with anti-llama IgG. A lack of L chain Abs was affirmed by coating with anti-llama IgG and detection with anti-κ or anti-λ Ig. Coating with anti-κ and, separately, anti-λ Ig and detection with anti-κ and anti-λ Ig, respectively, gave similarly negative results for the camμMT mice (data not shown). The low copy number line cam2μMT showed good levels of H chain IgG with no L chain. This line is represented with serum titrations of two mice (a and b, ~3-mo-old) chosen from four separate experiments with similar results, using serum from at least eight mice, each between 6 wk and 10 mo of age. Normal mouse serum (NM) showed some cross-reactivity.

pronounced by the presence of serum albumin and separation under reducing conditions, we coupled anti-llama IgG to Sepharose for the purification of H chain Abs. Bound Abs from serum were separated on 4–15% polyacrylamide gels and visualized with HRP-coupled anti-llama IgG. The results (Fig. 4*A*) showed under reducing conditions a major band of ~46 kDa in cam1μMT and cam2μMT mice. A second, fainter, band of ~64 kDa is only seen in cam2μMT mice. Cloning and sequencing of a 1.6-kb fragment, as compared with the normal 1.2-kb band, obtained from RT-PCR using V3FR1B (V_HH) and 3′C_HH3 oligos, revealed an aberrant splice product due to tandem integration of the construct, incorporating an extra V_HHDJ domain (supplemental Table I). This particular dromedary H chain is made up from V_HHDJ-V_HHDJ-H-C_HH2-C_HH3, which would add ~18 kDa to the normal size and explains the additional band obtained in Western analysis. However, this band is not a product using the C_H1 exon as verified by RT-PCR. The samples separated under reducing conditions showed faint H and L chain background bands from IgG coupled to Sepharose due to leakage (38). A reason could be that the different preparations of anti-llama Ig used for capture and visualization allowed some cross-detection, possibly enhanced by the sensitivity of the Western analysis. Separation of captured Ig under nonreducing conditions (Fig. 4*A*, *right*) revealed a major band of ~91 kDa, which represents H chain dimers. There are two larger bands, one of ~112 kDa and a much fainter band of ~135 kDa, which are likely to account for different multimers. Although the separation suggests that the secreted dromedary IgG2a H chain Ab produced in cam mice is largely associated as H2 homodimer, it may also associate as H3 multimer and, depending on resulting transcription products, in extended (2 × 64 kDa) or unequal (46 + 64 kDa) form. The longer exposure used to visualize H chain Ig products from cam1μMT mice is due to the lower levels produced (see Fig. 3).

Because no endogenous Ig could be identified in serum from camμMT mice, we conducted further Western separation on cam2 mice bred into the normal mouse background. Serum samples were applied to the gel in different amounts to allow a meaningful comparison. Figure 4*B* shows that no Igλ or Igκ could be detected in total cam2 serum, whereas significant amounts of dromedary H chain Ig remained as the only serum Ab. In summary, serum from

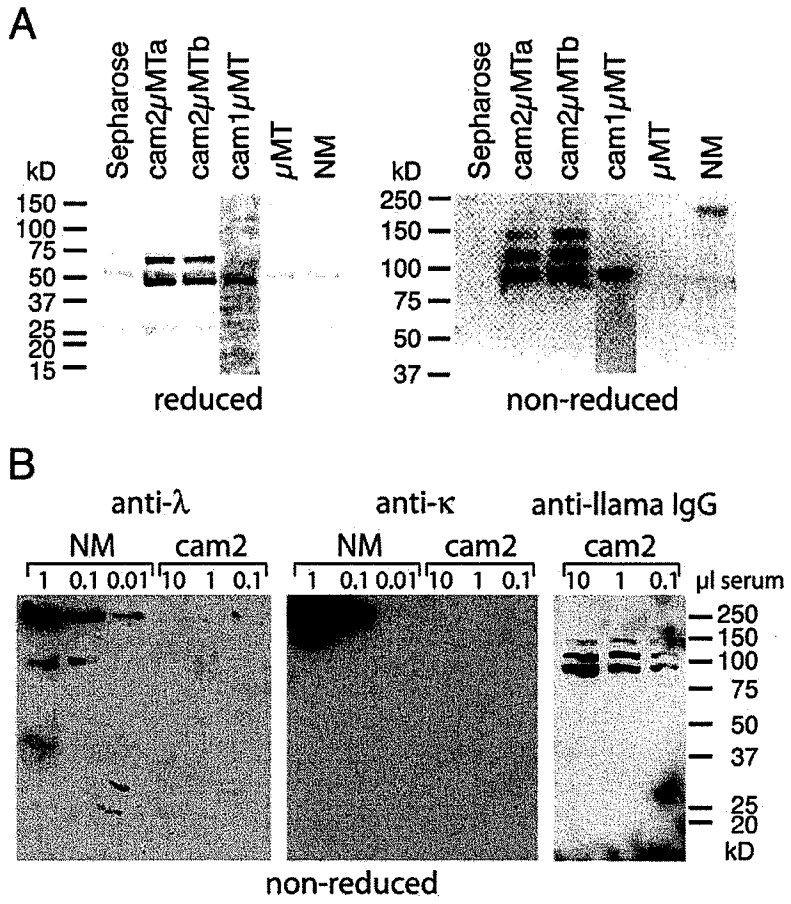


FIGURE 4. Western blot analysis of dromedary IgG2a. *A*, Serum Abs were captured by incubation with anti-llama IgG coupled to Sepharose, separated on Ready Gels, and stained with HRP-conjugated anti-llama IgG. Reducing conditions (*left*) revealed a ~46-kDa H chain band for cam2μMT (from mouse a and b) and cam1μMT (which was visible after longer exposure), and an additional band of ~64 kDa for cam2μMT. The fainter bands of ~26 kDa and ~53 kDa in all of the samples are the result of leakage of the Ab-coupled Sepharose (38) and cross-reactivity of different batches of anti-IgG, which contain IgG themselves. Samples separated under nonreducing conditions (*right*) revealed a major band of ~91 kDa (H2 configuration) for cam1μMT and cam2μMT. Additional bands found for cam2μMT of ~112 kDa and ~135 kDa may represent other multimers. Negative controls to affirm the specificity of the detection were anti-llama IgG-coupled Sepharose incubated with PBS, normal mouse (NM), and μMT serum. *B*, Total serum in 10-fold dilutions from cam2 in the normal mouse background and NM was separated under nonreducing conditions. Detection with anti-λ and anti-κ did not identify associated or residual L chain in cam2 mice, whereas development with anti-llama IgG showed the expected dromedary H chains. The filter was stripped between reactions. Detection with anti-κ necessitated a short exposure because of the strong NM signal. However, even longer exposures did not reveal any L chains in cam serum. The sizes of the marker bands are indicated.

camμMT or cam2 mice did not reveal any free or differently associated L chain by identification with anti-L chain reagents in ELISA and Western blotting, which showed that camelid H chain Ig can be exclusively produced in a mouse.

Progression of B cell development in bone marrow and spleen without L chain

A central question was how B cell development could progress in mice that express H chain Ig without L chain. Flow cytometry analysis of bone marrow cells (Fig. 5 left) from normal, μMT, and cam1μMT mice showed quite similar levels of pro- and pre-B cells, c-*kit*$^+$B220$^+$, and CD43$^+$B220$^+$, which are increased in cam2μMT mice (10% compared with 5% in normal mice or 4% in μMT mice). The reduced level of immature B220$^+$CD43$^-$ B cells in cam1μMT mice, 2% compared with 46% in a normal mouse, is barely detectable. However, cam2μMT mice, the low copy number but high expresser line, showed good recovery of the B lymphocyte population (14% compared with 46% B220$^+$ cells), which suggests induced B cell recovery by expression of the dromedary H chain gene. The effect is more dramatic in the spleen of cam2μMT mice (Fig. 5, *right*), where recovery of mature Ab-producing B cells reached about half the normal levels as shown in stainings with anti-CD21/35. Only poor recovery, 1.1%, is found in cam1μMT mice in concordance with the low level of expression found in this line. There is no expression of mouse IgM or Igκ or Igλ (data not shown) L chains. In the experiments, age-matched 3-mo-old mice were used. But very similar results were obtained, in flow cytometry, ELISA, Western, and PCR detection, when separate and parallel comparisons of 3-mo-, 6-wk-, and 6–10-mo-old animals were conducted. These results show that introduction of the dromedary H chain gene reconstitutes B cell development in the μMT or IgM-negative background without a requirement for L chain.

The H chain BCR signals exclusion of endogenous Ig

The purpose of crossing the dromedary H chain transgenic mice into the μMT background was to visualize H chain production without interference of mouse Ig. Initially, this was important, because the Abs that recognized the dromedary γ2a H chain cross-reacted with mouse Ig (see Figs. 3, and 4*A*). However, from the detailed analysis of the camμMT mice and serum Ig comparisons with cam2 mice bred into the normal mouse background, it became clear that dromedary H chain was well expressed and that B cell development progressed without L chain production. These results prompted further investigations to determine whether Ag-specific H chain Ig could be expressed on the cell surface and in the cytoplasm solely on its own or whether expression was accompanied by endogenous H or L chain polypeptides. Staining of bone marrow and spleen cells from cam2 transgenic mice with labeled HEL showed the presence of the Ag receptor on the cell surface of B220$^+$ cells (Fig. 6*A*). Binding of HEL was conducted in parallel using cells from MD-4 transgenic mice, which express HEL-specific Abs of high affinity (39). Although prominent surface staining with HEL was achieved in cam mice, the intensity was reduced compared with that of the MD-4 mice, perhaps due to low H chain density or reduced affinity. Receptor expression was not accompanied, even in the normal mouse background, by surface expression of endogenous IgM or IgL. Because this did not rule out the presence of endogenous Ig intracellularly, for example by chaperone retention, we used cytoplasmic staining. The results in Fig. 6*B*

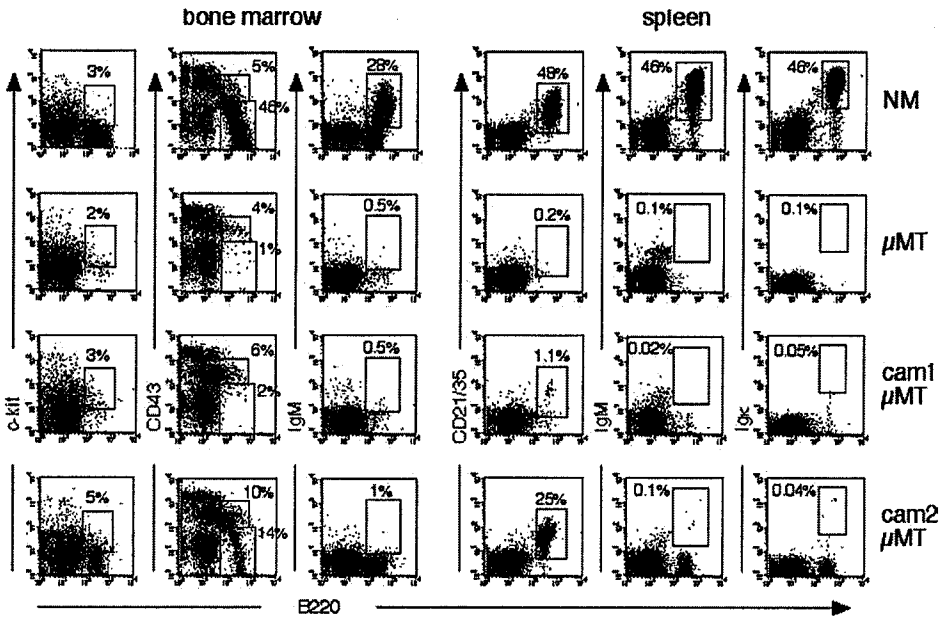


FIGURE 5. B cell development in mice expressing H chain-only Abs. Bone marrow (*left 3 rows*) and spleen cells (*right 3 rows*) from normal mice (NM), μMT, cam1μMT, and cam2μMT mice were stained with Abs against B cell differentiation markers. B220 served as a universal B cell marker and in combination with c-*kit*, and CD43 identified pro- and pre-B cells, which were well maintained in cam1μMT and cam2μMT mice. IgM is only expressed in normal mice, but cam2μMT mice showed good recovery of immature $B220^+$ cells, which are lacking in μMT and cam1μMT mice. Near normal levels of $CD21/35^+$ mature B cells were present in the spleen of cam2μMT mice (25%), but no L chain was found. The histograms were chosen from one of six independent experiments with very similar results, using ~3-mo-old mice.

showed HEL-specific Abs without the presence of mouse Ig. Unfortunately, some nonspecific background remains with this method (see *Materials and Methods*), which could point to residual mouse Ig expression at low levels. However, a similar background staining is found when using mice without endogenous C genes (3), reemphasizing that endogenous Ig levels are negligible.

H chain-only Abs are equally well expressed in C_H locus deletion mice

The findings that cam transgenic mice express dromedary H chain-only Ig in the absence of any mouse Ig chains and yet maintain appropriate levels of $B220^+$ cells, raised the question of whether endogenous H chain genes may be important for the early developmental stages. For example, pre-B cell development could be facilitated by VDJ recombination and endogenous H chain expression (μ, or δ in the μMT mice) initially accompanied but later replaced by transgene expression. It has been shown that the introduction of a rearranged murine γ transgene does not promote B cell development because joint expression with endogenous μ is required (40, 41). This finding suggested that IgG could not replace IgM, and it was speculated that their feedback signals to control B cell maturation must be different. To determine whether the dromedary γ2a H chain could be expressed without the help of other Ig genes, we crossed the cam2 mice with a recently derived line ($C\Delta^{-/-}$) where all constant region genes had been deleted (3). These animals cannot express any H chain isotypes. As can be seen in Fig. 6, *A* and *B*, cam2$C\Delta^{-/-}$ mice show the same level of B cells as cam2 mice and do not express any L chain. Indeed, it is worth noting that lymphocyte development, B cell levels, and Ab expression were very similar in cam$\mu MT^{-/-}$, cam, and

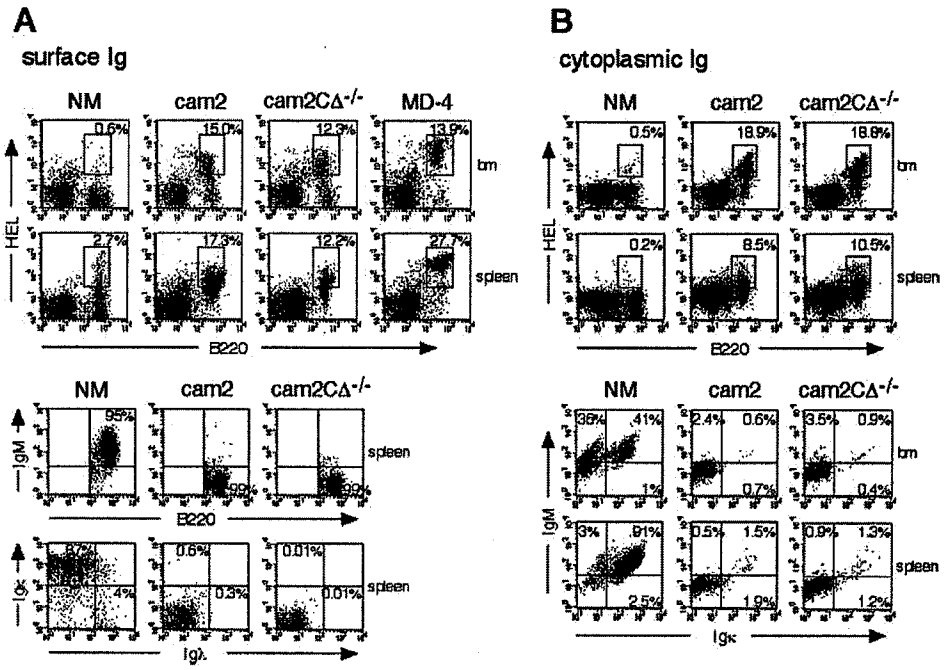


FIGURE 6. Make-up of the surface receptor and cytoplasmic Ig in bone marrow and spleen of cam2 mice in the normal mouse background and cam2$C\Delta^{-/-}$ mice with removed endogenous C_H locus. *A*, Identification of HEL-specific surface Ig plotted against B220 expression in normal mice (NM), cam2 mice, cam2$C\Delta^{-/-}$ and MD-4 (39) control mice (*top*), and stainings of μ-H and κ- and λ-L chain shown for the gated $B220^+$ lymphocytes (*below*). *B*, Cytoplasmic stainings confirmed the presence of HEL-specific Ig (*top*) and the lack of endogenous IgM and L chains in $B220^+$ cells from cam2 and cam2$C\Delta^{-/-}$ mice (*below*). The stainings show that bone marrow and splenic $B220^+$ cells from mice carrying the dromedary H chain transgene express HEL-specific Ig not accompanied by endogenous IgM or L chain.

camCΔ$^{-/-}$ mice, which established that the dromedary H chain transgene promotes B cell development independent of μ or expression of other endogenous H and L chains. Expression of HEL-specific H chain Abs on the cell surface suggests H chain association without L chain in dimeric or multimeric form. Such association presents a new type of BCR, with the capacity to initiate B cell development independent of conventional Ig expression.

Endogenous H and L chain loci are rearranged and transcribed

The lack of endogenous Ig expression suggested that the H chain BCR elicits the appropriate signals to prevent endogenous rearrangements. However, when analyzing bone marrow and spleen cell DNA from cam2 mice by semiquantitative PCR, we found, apart from the expected D-J_H bands, similar levels of V_HDJ_H and VκJκ rearrangement in cam2 and normal mice (Fig. 7). Using forward oligos representing different V_H gene families, *J558* and *7183*, gave a clear indication of diverse rearrangements in the cam2 mice, but we cannot completely rule out that endogenous V(D)J rearrangements are detected in B cells that have lost transgenic expression of the dromedary H chain. Hybridization with a full-length *J558* V_H gene further confirmed the similar levels of VDJ rearrangement in cam and normal mice. The obtained PCR fragments were of the expected sizes (3, 35). In the experiments, DNA from RAG2$^{-/-}$ tissues, used as a negative control, showed some background amplification. Such background was not seen when, for example, mouse embryonic stem cell DNA was used for V_HDJ_H amplification (data not shown). Different RAG2$^{-/-}$ mice sources and DNA preparations did not prevent this but always showed a much reduced signal compared with cam2 and normal mouse DNA. However, because we did not see any background in RT-PCR using RAG2$^{-/-}$ RNA, this could indicate a low level of nonproductive recombination products. Using lamin B1 as reference (3) for the semiquantitative comparison using serial dilutions, we found no indication of reduced levels in the cam mice. Control reactions conducted in parallel using normal mouse DNA for dromedary H chain amplification and dromedary *V_HH-Cγ2aTM* plasmid DNA for mouse V(D)J PCR did not result in nonspecific bands (data not shown).

To our surprise, RT-PCR signals, reflecting RNA levels, were also very similar in cam2 and normal mice. To assess whether the V558-J_H RT-PCR bands from cam2 mice accounted for nonfunctional VDJ rearrangements or represented potentially productive transcripts, we cloned and sequenced the ~400-bp fragments (supplemental Table II). Sequence comparison established that fully functional and diverse murine V_HDJ_H transcripts were produced in cam2 mice. To investigate whether endogenous transcripts were only expressed in cells that did not produce dromedary H chain Ig or whether endogenous and exogenous transcription was jointly operative in the same cell, we separated B220$^-$ and B220$^+$HEL$^+$ lymphocytes by flow cytometry (Fig. 8). Semiquantitative RT-PCR analysis of B220$^+$HEL$^+$ bone marrow and spleen cell RNA from cam2 mice showed extensive V558-J_H and Vκ-Cκ amplification similar to those from normal mice. In B220$^-$ cells, V(D)J transcripts were also well maintained, and certainly in the cam mice, there were no amplification differences. As this raised the possibility that the calculated purity, >95%, of the sorted cell populations may not have been reached, we used further RT-PCR to identify surrogate L chain transcripts. With VpreB and λ5, surrogate L chain polypeptides are well expressed in B220$^+$ bone marrow cells, but no expression is found in the spleen (Refs. 9, 11, 42, and refs. therein). This was exactly what we found and provided reassurance of the purity of the analyzed cell populations. A comparison of sorted B220$^+$ and B220$^-$ bone marrow and spleen cells from cam2 and normal mice showed no difference in transcription levels of the surrogate L chain (Fig. 8). Expression of surrogate L chain in bone marrow but not spleen B220$^+$ lymphocytes from cam mice was independently confirmed by cytoplasmic staining with anti-λ5 (data not shown). Control reactions (Fig. 8*C*) using cDNA prepared from bone marrow and spleen cells of RAG2$^{-/-}$ mice, bone marrow cells from SL (surrogate L chain triple knockout (KO))$^{-/-}$ mice (42), and DNA from normal mouse spleen cells confirmed the validity of the RT-PCR. The lack of VpreB and λ5 transcription in mature cam2 B cells rules out that dromedary H chain expression relies on the presence of surrogate L chain.

Our comprehensive analysis of intra- and extracellular expression of endogenous murine Ig revealed that very small amounts, if any, were retained in the cell. This may mean that either no translational products were being produced or that there was rapid degradation. Staining with anti-L chain confirmed a lack of endogenous Ig. In addition, we did not identify dromedary H chain transcripts in other nonlymphocyte tissues (data not shown). In

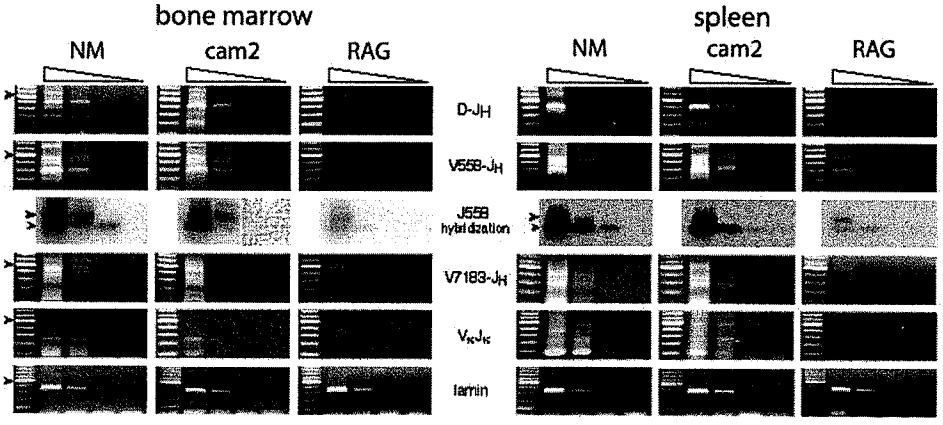


FIGURE 7. DNA rearrangement of endogenous *H* and *L* chain genes is maintained in cam mice. V(D)J recombination in bone marrow and spleen cells from normal mice (NM) and cam2 mice was examined by semiquantitative PCR using combinations of D-J_H, V558-J_H, V7183-J_H, and Vκ-Jκ primers. The DNA starter concentration was ~10 ng followed by three further dilutions, 10^{-1}, 10^{-2}, and 10^{-3}, each. RAG2$^{-/-}$ mice served as a negative control, and amplification of lamin B1 permitted normalization. Hybridization of blotted V558-J_H amplifications with a *J558* V_H gene probe confirmed the specificity of the reactions. Bands of the expected approximate sizes, 400–800 bp for V_HDJ_H and 200–400 for $V_\kappa J_\kappa$, depending on J segment usage (3, 35), were maintained in the cam mice. As a size marker, 100-bp ladders were used with the 600-bp band of increased intensity marked by an arrow, followed by a 100-bp size increase above and decrease below. For the J558 hybridization, 600 bp is marked by a normal arrow, and 400 bp is marked by a small arrow.

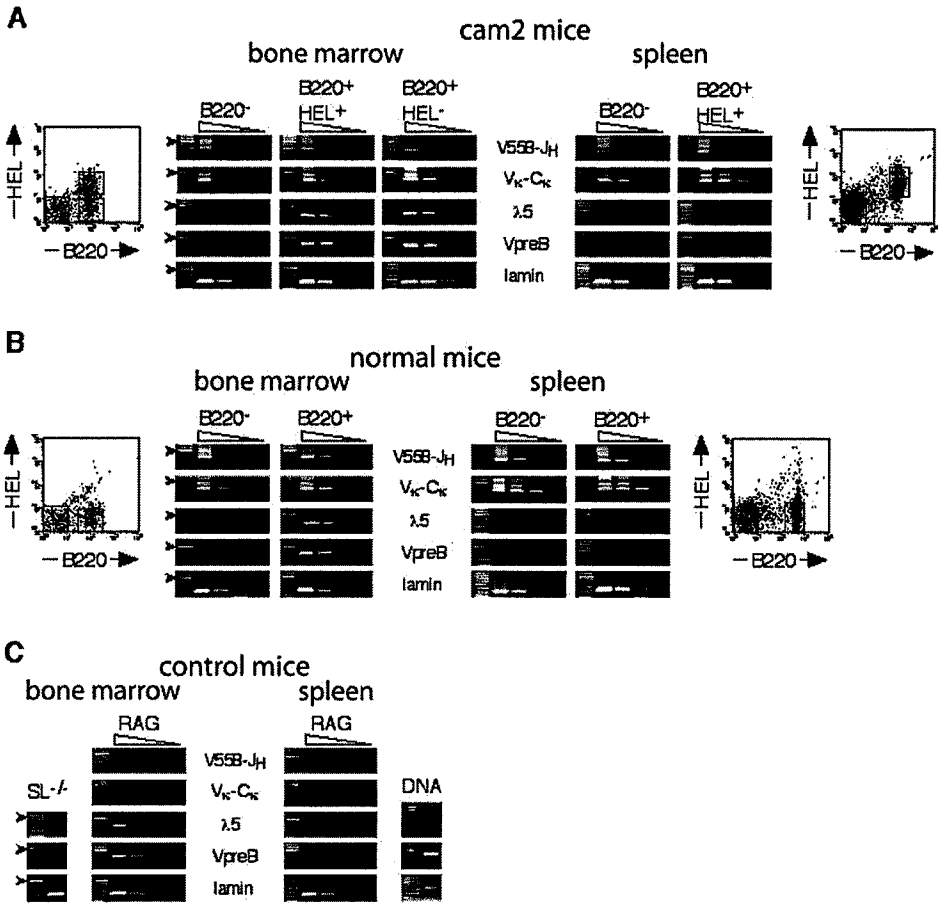


FIGURE 8. V(D)J transcription is operative in cam mice. Bone marrow and splenic lymphocytes from cam2 and normal mice, stained for B220 and HEL binding, were sorted by flow cytometry to >95% purity. RNA was produced from 10^5–10^6 sorted cells, and equal amounts of cDNA from ~5×10^3 cells were analyzed in parallel by PCR in successive 10-fold dilutions. Oligo combinations of V558-J_H and V_κ-C_κ determined the level of Ig transcription, λ5 and VpreB examined the developmental stage of the cell populations, and lamin provided an independent reference of the cDNA concentrations. *A*, RT-PCR analysis of gated bone marrow and spleen cell populations, $B220^-$, $B220^+$, and $B220^+HEL^+$, from cam2 mice. *B*, $B220^-$, $B220^+$ cells from normal mice. *C*, Control reactions using RT-PCR analysis using tissue from surrogate L chain KO mice (42) and $RAG2^{-/-}$ mice, and PCR using normal DNA, which confirmed the size difference of the genomic products.

conclusion, this suggests that cam transgenic mice express the dromedary H chain-only Ab without association or attendance of significant amounts of endogenous Ig. Nevertheless, it may be possible that the transgene can only be expressed after endogenous V(D)J rearrangement has been completed. However, dromedary H chain expression in $C\Delta^{-/-}$ mice, which do not express rearranged H chains without C region (3), rules out that B cell differentiation is driven by endogenous IgH expression. The results show that endogenous H and L chain genes, despite being fully rearranged and transcribed, are excluded from expression at the translational stage by a feedback signal originating from a H chain BCR without L chain.

Discussion

Introduction of a rearranged dromedary *H* chain gene into the mouse germline showed that Ag-specific H chain-only Abs could be correctly expressed, without the C_H1 domain, and assembled as multimer. H chain Ig was secreted and also presented on the cell surface, which led to progression in B cell development. Expression of H chain IgG might exclude translation of endogenous H and L chain polypeptides, which established a BCR without L chain association.

The rearranged H chain expressed in transgenic mice was constructed with no alteration that would favorably bias expression in mouse B cells. Thus, secretion and surface expression of HEL-specific H chain Abs in a heterologous system established that RNA processing, H chain assembly, and cellular transport use commonly recognized signals provided by the dromedary V_HH-γ2aTM construct. The likely reason why dromedary H chain Ig can be expressed in the mouse seems to be due to two gene adaptations in camelids, not found in other jawed vertebrates. Their V_HH genes are distinct from conventional V_H genes; they accommodate changes in key residues normally in contact with the V_L domain in the Ag binding site of conventional Abs (16). Apparently, neither the V_HH hallmark amino acids, nor the presence of a long CDR3 loop of 24 aa, caused folding problems (43). Nevertheless, the genomic organization of the V_HH genes (i.e., promoter, leader signal, intron, V-exon, and recombination signal sequence) is otherwise remarkably similar to that of the conventional V_H counterparts (44). It has been reasoned that V_HH genes have recently evolved from conventional V_H genes after the emergence of the Tylopoda (>50 million years ago), which makes it likely that both types are accommodated in the *V* gene cluster of the *H* chain locus (44). This is supported by the observation that both the V_H and V_HH gene segments appear to rearrange to the same *D* and J_H gene segments to form either a conventional Ab or a H chain Ab (21). The other adaptation concerns a subset of their *Cγ* genes (24, 25). It was proposed that in these genes, a point mutation at the canonical splice signal sequence might cause the excision of the first C region domain (24). Although the precise mechanism is not known, this removal seems to permit assembly and secretion of homodimeric H chains (20). Interestingly, accurate and highly efficient removal of the C_H1-containing sequence from the RNA transcript of *H* chain genes appears to be performed with equal efficiency in camelids and transgenic mice. Neither in the dromedary nor in our transgenic mice could *γ2a H* chain genes with retained C_H1 exon be identified by RT-PCR and sequencing. Thus, the removal of the C_H1 exon appears to be essential to permit expression of H chain Ig. However, exclusive H chain-only Ab production in camelids was predicted to involve interaction with

species-specific cellular factors important for the expression of H chain Ab genes, processing of their transcripts, and the assembly of the translation products into functional Ag binding entities (45). For this reason, it was unexpected to see that a heterologous system produced functional H chain Abs at quite respectable protein levels. This suggested that intrinsic alterations of the dromedary H chain Ab are well recognized and dealt with by the mouse B cells and that dromedary-specific factors are either not essential or can be bypassed by the mouse transcription, translation, and secretion machinery.

The C_H1 domain participates actively in the regulation of the assembly and secretion of conventional H2L2 Abs via association with BiP (26–29). A lack of C_H1 is likely to permit unhindered transit of the H chain polypeptide through the endoplasmic reticulum to allow secretion and appropriate surface deposition. Furthermore, the loss of BiP association may also prevent degradation of the H chain. H chains with the long hydrophobic transmembrane region anchor in the lipid bilayer, whereas the short hydrophilic C-terminal region of secretory form H chains ensures their release from the cell in the absence of associated BiP. The importance of the C_H1 domain is well recognized because hybridoma or myeloma cell lines harboring Ig genes with deleted C_H1 exon retain the ability to secrete homodimeric H chains without associated L chains (46, 47). In heavy chain disease, truncated H chains are readily secreted without L chain (17, 48). For the dromedary H chain, not being dependent on IgM expression may allow the expansion of a different lymphocyte subset, which may be able to restore normal B cell development. Extensive levels of $B220^+$ cells, some with dendritic cell characteristics, have been found in bone marrow and spleen (49) and may be maintained in the dromedary H chain mice. Alternatively, expression of the rearranged dromedary H chain gene could facilitate progression in B cell development to a mature stage without the differentiation stages from pro- to pre-B cells ($B220^+CD43^-$ cells in Fig. 5). In this context, it is notable that staining of camel lymphocytes for Ig H and L chain on the cell surface has been attempted but did not unambiguously demonstrate surface IgG H chain-only expression. A reason for this may be that the staining reagents raised against ruminant Ig fail if there is broad epitope diversity (50). Despite this setback, camelids readily produce Ag-specific Abs in H2 and H2L2 configuration, and there is no indication that mixed molecules are expressed (16, 51). Unfortunately, there is no information about pre-B cell development in camelids or whether an H chain without C_H1 can associate with a surrogate L chain to form the pre-BCR necessary to progress B cell development. However, from gene targeting studies in the mouse, it is clear that B cell development without surrogate L chain can progress (42), whereas B cell development without L chain is blocked after H chain expression and maturation up to the immature B cell stage (4).

The various Ig classes seem to form distinct oligomeric BCR complexes, which may differ in their threshold levels for BCR signaling (Ref. 52, and refs. therein). For example, the IgG BCR complex, in contrast to the IgM or IgD BCR complex, cannot give an efficient positive selection signal. Perhaps, contradictory to expectation, the H chain BCR may be able to provide an adequate differentiation and proliferation signal to secure survival. In transgenic mice carrying rearranged conventional *H* chain genes (μ, δ, γ, or α), feedback inhibition can prevent DNA rearrangement of the endogenous *IgH* locus (References 53–55, and references therein). However, the expression of the transmembrane form of introduced Ig transgenes does not necessarily prevent DNA rearrangement of the endogenous loci to secure allelic exclusion (56–58). It has also been shown that *γ2b* transgenes are coexpressed with endogenous μ, and that *γ2b* cannot by itself promote B cell development in the μ KO background (40, 55, 59). The few mature B cells that do develop in the transgenic mice express both endogenous μ and transgenic γ2b, and in addition, L chain is expressed. Although these experiments show that B cell development is critically dependent on signaling of a μ-H chain associated as BCR, there are exceptions. In a particular *γ2b* transgenic mouse line, it appears that transgene expression by itself can promote B cell maturation and allelic exclusion, possibly by expanding a particular B cell subset (55). In separate founders, most likely carrying a γ2b transgene integration at diverse chromosomal locations, it was discovered that alternative expression pathways were used, maybe dictated by different expression levels. Despite these contradictory results, which may largely depend on site of integration and copy number of particular *IgH* transgenes, the overall conclusion from transgenic IgH mouse studies is that B cell maturation can progress, but the developmental state of the lymphocytes appears to be critically dependent on the onset of (endogenous) μ expression. This was not seen in the cam mice, where even in a normal mouse background, IgM expression was prevented without causing developmental cessation.

The two cam transgenic lines we describe in this study, derived from independent microinjections, are most likely to carry the transgenes at different chromosomal sites. Despite this and the low H chain expression level in cam1 mice, developmental progression is very similar. The recent finding that, in the mouse, entire μ-H chains can be transported and expressed on the surface of pre-B cells without associated L chains (9–11) contradicts previous discussions that free H chain polypeptides are toxic and that they have to be neutralized to allow progression in B cell development (Ref. 60, and refs. therein). The observations may be compatible if lower H chain expression levels in early B cells are taken into account and if apoptosis, which may be induced when insoluble (accumulated) H chain complexes damage the cell, occurs at a later differentiation stage. The lack of L chain in the cam mice must be the result of the failure of the dromedary H chains to associate with L chains, which arises from the difference in important residues in V_HH genes compared with V_H genes (44). Prohibited L chain association may act as a feedback signal that stops L chain translation. This would be in agreement with the observation that in healthy individuals, H chain expression balances L chain synthesis to accomplish equimolar levels (61). In the dromedary H chain mice, which are perfectly healthy, the induced lack of L chain may prevent expression of endogenous H chains, which, if not removed, could be toxic for the cell. In addition, this emphasizes that the introduced dromedary H chain appears to be fully active in securing allelic feedback, albeit at the very late translational stage, which still allows productive DNA rearrangements and transcription of potentially functional endogenous H chains.

The presence of the BCR is essential to govern B cell survival and differentiation (62). Thus, H chain Ab deposition on the cell surface is of key importance for the formation of the H chain Ab repertoire (63, 64). The formation of H chain Abs in camelids is decided by rearrangement of a V_HH gene to commonly used D and J_H segments (44) and (switch?) recombination to a $C\gamma$ gene that permits the removal of C_H1 (22, 24). We speculate that transitory surface expression of μ-H chain without L chain association, as described in the mouse (9–11), may also occur in camelids, and perhaps, unlike in mice, may facilitate successful switching and expression of H chain IgG isotypes with their particular *V* genes, which do not tolerate association with L chain (15, 16, 23). Expression of the membrane form strongly suggests the presence of memory B cells for H chain Abs in camelids. Such cells would undergo an Ab maturation process, leading to H chain Abs with

improved affinities for the Ag. The finding of extensive diversification of H chain Abs (21), but the failure to detect an IgM isotype without L chains in camelids (22, 23), has unexpected implications for H chain Ab ontogeny because it questions the involvement of μ^+ B cells bearing conventional IgM as precursors of H chain Ab-producing cells. For this reason, it becomes important to reassess the developmental progression of B lymphocytes, which can express H chain Abs. The successful generation of transgenic H chain Abs paves the way for the creation of single-chain repertoires in the mouse by introduction of modified V_H genes and splice site mutation in a γ-H chain gene. Retention of V gene variability allows recognition of novel epitopes regarded as poorly accessible by conventional Abs and provides the advantage that single-chain binders are not dependent on successful V_H/V_L pairing (16).

Acknowledgments

We thank P. Kilshaw, M. Neuberger, and A. Corcoran for helpful comments; S. Bell and E. Vigorito for advice and control reagents; S. Bunting and A. Wood for the provision of oligos; C. Mundt and L. Martensson for control tissue; S. Davies for providing RAG2 KO mice; and G. Morgan for help with the flow cytometry.

Disclosures

The authors have no financial conflict of interest.

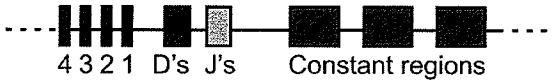
References

1. Kearny, J. F. 2004. Development and function of B-cell subsets. In *Molecular Biology of B Cells*, T. Honjo, F. W. Alt, and M. S. Neuberger, eds. Elsevier Academic Press, Amsterdam, p. 155–160.
2. Chen, J., M. Trounstine, F. W. Alt, F. Young, C. Kurahara, J. F. Loring, and D. Huszar. 1993. Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the J_H locus. *Int. Immunol.* 5: 647–656.
3. Ren, L., X. Zou, J. A. Smith, and M. Brüggemann. 2004. Silencing of the immunoglobulin heavy chain locus by removal of all 8 constant region genes on a 200 kb region. *Genomics* 84: 686–695.
4. Zou, X., T. A. Piper, J. A. Smith, N. D. Allen, J. Xian, and M. Brüggemann. 2003. Block in development at the pre-B-II to immature B cell stage in mice without Igκ and Igλ light chain. *J. Immunol.* 170: 1354–1361.
5. Zou, X., C. Ayling, J. Xian, T. A. Piper, P. J. Barker, and M. Brüggemann. 2001. Truncation of the μ heavy chain alters BCR signalling and allows recruitment of $CD5^+$ B cells. *Int. Immunol.* 13: 1489–1499.
6. Corcos, D., A. Iglesias, O. Dunda, D. Bucchini, and J. Jami. 1991. Allelic exclusion in transgenic mice expressing a heavy chain disease-like human μ protein. *Eur. J. Immunol.* 21: 2711–2716.
7. Shaffer, A. L., and M. S. Schlissel. 1997. A truncated heavy chain protein relieves the requirement for surrogate light chains in early B cell development. *J. Immunol.* 159: 1265–1275.
8. Muljo, S. A., and M. S. Schlissel. 2002. The variable, C_H1, C_H2 and C_H3 domains of Ig heavy chain are dispensable for pre-BCR function in transgenic mice. *Int. Immunol.* 14: 577–584.
9. Su, Y., A. Flemming, T. Wossning, E. Hobeika, M. Reth, and H. Jumaa. 2003. Identification of a pre-BCR lacking surrogate light chain. *J. Exp. Med.* 198: 1699–1706.
10. Schuh, W., S. Meister, E. Roth, and H.-M. Jäck. 2003. Cutting edge: signaling and cell surface expression of a μH chain in the absence of λ5: a paradigm revisited. *J. Immunol.* 171: 3343–3347.
11. Galler, G. R., C. Mundt, M. Parker, R. Pelanda, I.-L. Mårtensson, and T. H. Winkler. 2004. Surface heavy chain signals down-regulation of the V(D)J-recombinase machinery in the absence of surrogate light chain components. *J. Exp. Med.* 199: 1523–1532.
12. Padlan, E. A. 1994. Anatomy of the antibody molecule. *Mol. Immunol.* 31: 169–217.
13. Litman, G. W., M. K. Anderson, and J. P. Rast. 1999. Evolution of antigen binding receptors. *Annu. Rev. Immunol.* 17: 109–147.
14. Hamers-Casterman, C., T. Atarhouch, S. Muyldermans, G. Robinson, C. Hamers, E. B. Songa, N. Bendahman, and R. Hamers. 1993. Naturally occurring antibodies devoid of light chains. *Nature* 363: 446–448.
15. Muyldermans, S., T. Atarhouch, J. Saldanha, J. A. Barbosa, and R. Hamers. 1994. Sequence and structure of VH domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. *Protein Eng.* 7: 1129–1135.
16. Muyldermans, S., C. Cambillau, and L. Wyns. 2001. Recognition of antigens by single-domain antibody fragments: the superfluous luxury of paired domains. *Trends Biochem. Sci.* 26: 230–235.
17. Alexander, A., M. Steinmetz, D. Barritault, B. Frangione, E. C. Franklin, L. Hood, and J. N. Buxbaum. 1982. γ Heavy chain disease in man: cDNA sequence supports partial gene deletion model. *Proc. Natl. Acad. Sci. USA* 79: 3260–3264.
18. Greenberg, A. S., D. Avila, M. Hughes, A. Hughes, E. C. McKinney, and M. F. Flajnik. 1995. A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. *Nature* 374: 168–173.
19. Rast, J. P., C. T. Amemiya, R. T. Litman, S. J. Strong, and G. W. Litman. 1998. Distinct patterns of IgH structure and organization in a divergent lineage of chrondrichthyan fishes. *Immunogenetics* 47: 234–245.
20. Nguyen, V. K., C. Su, S. Muyldermans, and W. van der Loo. 2002. Heavy-chain antibodies in Camelidae: a case of evolutionary innovation. *Immunogenetics* 54: 39–47.
21. Nguyen, V. K., R. Hamers, L. Wyns, and S. Muyldermans. 2000. Camel heavy-chain antibodies: diverse germline V_HH and specific mechanisms enlarge the antigen-binding repertoire. *EMBO J.* 19: 921–930.
22. Nguyen, V. K., A. Desmyter, and S. Muyldermans. 2001. Functional heavy-chain antibodies in Camelidae. *Adv. Immunol.* 79: 261–296.
23. Nguyen, V. K. 2002. Generation of heavy chain antibodies in camelids. Doctor of Philosophy thesis, Free University of Brussels, Brussels, Belgium.
24. Nguyen, V. K., R. Hamers, L. Wyns, and S. Muyldermans. 1999. Loss of splice consensus signal is responsible for the removal of the entire C_H1 domain of the functional camel IGG2A heavy-chain antibodies. *Mol. Immunol.* 36: 515–524.
25. Woolven, B. P., L. G. Frenken, P. van der Logt, and P. J. Nicholls. 1999. The structure of the llama heavy chain constant genes reveals a mechanism for heavy-chain antibody formation. *Immunogenetics* 50: 98–101.
26. Haas, I. G., and M. Wabl. 1983. Immunoglobulin heavy chain binding protein. *Nature* 306: 387–389.
27. Munro, S., and H. R. Pelham. 1987. A C-terminal signal prevents secretion of luminal ER proteins. *Cell* 48: 899–907.
28. Hendershot, L., D. Bole, G. Köhler, and J. F. Kearney. 1987. Assembly and secretion of heavy chains that do not associate posttranslationally with immunoglobulin heavy chain-binding protein. *J. Cell Biol.* 104: 761–767.
29. Hendershot, L. M. 1990. Immunoglobulin heavy chain and binding protein complexes are dissociated in vivo by light chain addition. *J. Cell Biol.* 111: 829–837.
30. Lauwereys, M., M. A. Ghahroudi, A. Desmyter, J. Kinne, W. Holzer, E. De Genst, L. Wyns, and S. Muyldermans. 1998. Potent enzyme inhibitors derived from dromedary heavy-chain antibodies. *EMBO J.* 17: 3512–3520.
31. Nguyen, V. K., X. Zou, M. Lauwereys, L. Brys, M. Brüggemann, and S. Muyldermans. 2003. Heavy-chain only antibodies derived from dromedary are secreted and displayed by mouse B cells. *Immunology* 109: 93–101.
32. Hogan, B., R. Beddington, F. Costantini, and E. Lacy. 1994. Production of transgenic mice. In *Manipulating the Mouse Embryo: A Laboratory Manual.* Cold Spring Laboratory Press, Cold Spring Harbor, p. 217–252.
33. Kitamura, D., J. Roes, R. Kuhn, and K. Rajewsky. 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. *Nature* 350: 423–426.
34. Sambrook, J., and D. W. Russell. 2001. *Molecular Cloning*, 3rd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
35. Hertz, M., and D. Nemazee. 1997. BCR ligation induces receptor editing in IgM^+IgD^- bone marrow B cells in vitro. *Immunity* 6: 429–436.
36. Zou, X., J. Xian, A. V. Popov, I. R. Rosewell, M. Müller, and M. Brüggemann. 1995. Subtle differences in antibody responses and hypermutation of λ light chains in mice with a disrupted κ constant region. *Eur. J. Immunol.* 25: 2154–2162.
37. Bruce, S. R., R. W. C. Dingle, and M. L. Peterson. 2003. B-cell and plasma-cell splicing differences: a potential role in regulated immunoglobulin RNA processing. *RNA* 9: 1264–1273.
38. Sato, H., T. Kidaka, and M. Hori. 1987. Leakage of immobilized IgG from therapeutic immunoadsorbents. *Appl. Biochem. Biotechnol.* 15: 145–158.
39. Brink, R., C. C. Goodnow, J. Crosbie, E. Adams, J. Eris, D. Y. Mason, S. B. Hartley, and A. Basten. 1992. Immunoglobulin M and D antigen receptors are both capable of mediating B lymphocyte activation, deletion, or anergy after interaction with specific antigen. *J. Exp. Med.* 176: 991–1005.
40. Roth, P. E., L. Doglio, J. T. Manz, J. Y. Kim, D. Lo, and U. Storb. 1993. Immunoglobulin γ2b transgenes inhibit heavy chain gene rearrangement, but cannot promote B cell development. *J. Exp. Med.* 178: 2007–2021.
41. Schlissel, M. S., and T. Morrow. 1994. Ig heavy chain protein controls B cell development by regulating germ-line transcription and retargeting V(D)J recombination. *J. Immunol.* 153: 1645–1657.
42. Shimizu, T., C. Mundt, S. Licence, F. Melchers, and I. L. Martensson. 2002. VpreB1/VpreB2/λ5 triple-deficient mice show impaired B cell development but functional allelic exclusion of the IgH locus. *J. Immunol.* 168: 6286–6293.
43. Desmyter, A., T. R. Transue, M. A. Ghahroudi, M. H. Thi, F. Poortmans, R. Hamers, S. Muyldermans, and L. Wyns. 1996. Crystal structure of a camel single-domain VH antibody fragment in complex with lysozyme. *Nat. Struct. Biol.* 3: 803–811.
44. Nguyen, V. K., S. Muyldermans, and R. Hamers. 1998. The specific variable domain of camel heavy-chain antibodies is encoded in the germline. *J. Mol. Biol.* 275: 413–418.
45. Conrath, K. E., U. Wernery, S. Muyldermans, and V. K. Nguyen. 2003. Emergence and evolution of functional heavy-chain antibodies in Camelidae. *Dev. Comp. Immunol.* 27: 87–103.
46. Sitia, R., M. Neuberger, C. Alberini, P. Bet, A. Fra, C. Valetti, G. Williams, and C. Milstein. 1990. Developmental regulation of IgM secretion: the role of the carboxy-terminal cysteine. *Cell* 60: 781–790.
47. Morrison, S. L. 1978. Murine heavy chain disease. *Eur. J. Immunol.* 8: 194–199.
48. Witzig, T. E., and D. L. Wahner-Roedler. 2002. Heavy chain disease. *Curr. Treat. Options Oncol.* 3: 247–254.
49. Nikolic, T., G. M. Dingjan, P. J. M. Leenen, and R. W. Hendriks. 2002. A subfraction of $B220^+$ cells in murine bone marrow and spleen does not belong to

the B cell lineage but has dendritic cell characteristics. *Eur. J. Immunol.* 32: 686–692.
50. Ungar-Waron, H., R. Yagil, J. Brenner, R. Paz, N. Partosh, C. Van Creveld, E. Lubashevsky, and Z. Trainin. 2003. Reactions of peripheral blood mononuclear cells (PBMC) of camels with monoclonal antibodies against ruminant leukocytes. *Comp. Immunol. Microbiol. Infect. Dis.* 26: 137–143.
51. van der Linden, R., B. de Geus, W. Stok, W. Bos, D. van Wassenaar, T. Verrips, and L. Frenken. 2000. Induction of immune responses and molecular cloning of the heavy chain antibody repertoire of *Lama glama*. *J. Immunol. Methods* 240: 185–195.
52. Reth, M. 2001. Oligomeric antigen receptors: a new view on signaling for the selection of lymphocytes. *Trends Immunol.* 22: 356–360.
53. Weaver, D., F. Costantini, T. Imanishi-Kari, and D. Baltimore. 1985. A transgenic immunoglobulin μ gene prevents rearrangement of endogenous genes. *Cell* 42: 117–127.
54. Nussenzweig, M. C., A. C. Shaw, E. Sinn, D. B. Danner, K. L. Holmes, H. C. Morse, III, and P. Leder. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin μ. *Science* 236: 816–819.
55. Kenny, J. J., A. M. Stall, R. T. Fisher, E. Derby, M. C. Yang, P. W. Tucker, and D. L. Longo. 1995. Igγ2b transgenes promote B cell development but alternate developmental pathways appear to function in different transgenic lines. *J. Immunol.* 154: 5694–5705.
56. Stall, A. M., F. G. Kroese, F. T. Gadus, D. G. Sieckmann, L. A. Herzenberg, and L. A. Herzenberg. 1988. Rearrangement and expression of endogenous immunoglobulin genes occur in many murine B cells expressing transgenic membrane IgM. *Proc. Natl. Acad. Sci. USA* 85: 3546–3550.
57. Forni, L. 1990. Extensive splenic B cell activation in IgM-transgenic mice. *Eur. J. Immunol.* 20: 983–989.
58. Yuan, D., and T. Dang. 1997. Effect of the presence of transgenic *H* and *L* chain genes on B cell development and allelic exclusion. *Int. Immunol.* 9: 1651–1661.
59. Kurtz, B. S., P. L. Witte, and U. Storb. 1997. γ2b provides only some of the signals normally given via μ in B cell development. *Int. Immunol.* 9: 415–426.
60. Haas, I. G., and M. R. Wabl. 1984. Immunoglobulin heavy chain toxicity in plasma cells is neutralized by fusion to pre-B cells. *Proc. Natl. Acad. Sci. USA* 81: 7185–7188.
61. Wall, R., and M. Kuehl. 1983. Biosynthesis and regulation of immunoglobulins. *Annu. Rev. Immunol.* 1: 393–422.
62. Williams, G. T., P. Dariavach, A. R. Venkitaraman, D. J. Gilmore, and M. S. Neuberger. 1993. Membrane immunoglobulin without sheath or anchor. *Mol. Immunol.* 30: 1427–1432.
63. Lam, K. P., R. Kühn, and K. Rajewsky. 1997. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell* 90: 1073–1083.
64. Torres, R. M., H. Flaswinkel, M. Reth, and K. Rajewsky. 1996. Aberrant B cell development and immune response in mice with a compromised BCR complex. *Science* 272: 1804–1808.

immunizecamelid with a specific antigen

VHH's embedded among VH segments, rest of structure of the camelid loci is not known



VDJ rearrangement e.g. VHH3, it is not known how class switching occurs

hypermutation in periphery, i.e affinity maturation for specific antigen and camelid class switching

transcription in camelid B cells

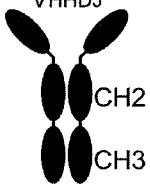
cDNA

RNA

translation in camelid B cells

cloneVHHDJ in expression vector

VHHDJ



HCAb from camelid

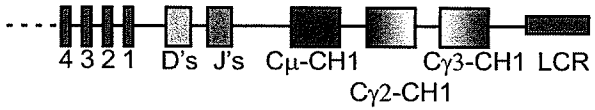
introduce into transgenic mice

transcription and translation in transgenic mouse B or other cells yields a camelid VHHDJ (no effector CH2/CH3) same sequence as in the camelid cDNA VHHDJ above

VHHDJ

LEDBETTER

heavy chain only camelid antibody diversity and selection for specific antigen response carried out in the camel or llama



generate a (human) immunoglobulin locus with VH segment, D segments,J segments and constant regions lacking CH1

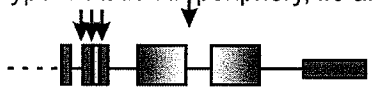
introduce into transgenic mice preferably without a functional endogenous murine immunoglobulin heavy chain locus, e.g μMT

transgenic mouse

immunize

VDJ rearrangement (e.g. VH4) of transgenic locus

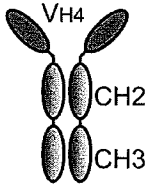
hypermutation in periphery, i.e affinity maturation for specific antigen and class switching (e.g. to Cγ2)



transcription in mouse B cells

RNA

translation in mouse B cells



newly generated (human) HCAb (IgG)

THIS PATENT (JANSSENS ET AL)

heavy chain only (any species) antibody diversity and selection for specific antigen response carried out in transgenic mouse